

SI Appendix

V_H alignment

V _H alignment	1	26	33	51	57	93	
	CDR1			CDR2		CDR3	
IGHV3-23*01	EVQLLESGGGLVQPGGSLRLSCAAS	GFTFS	SYAMSWVRQAPGKGLEWVSA	ISG	SGGSTYYADSVKGRFTISRDNSKNTLYLQMN	SLRAEDTAVYYCAK	-----
IGHD3-10*01	-----	-----	-----	-----	-----	VLLWFG	LI-----
IGHJ4*02	-----	-----	-----	-----	-----	YFDI	WGQGLTVTVSS
MEX18_07	EVQLLESGGGLVQPGGSLRLSCAAS	GFTFS	SYAMNWSVRQAPGKGLEWVSG	IGGRGAIAGDGS	IYYADSVKGRFTISRDNSKNTLYLQMN	GLRVEDTAVYYCAK	DR-----VAFDPGHVWGQGLTVTVSS
MEX18_15	EVQLLESGGGLVQPGGSLRLSCAS	GFTFS	SYAMSWVRQAPGKGLEWVSG	ISP	LDGSTYYAASVKGRFTISRDNSKNTLYLQMN	SLRVEDTAVYYCAK	DRITMGVGE-LFVWVGQGLTVTVSS
MEX18_21	EVQLLESGGGLVQPGGSRRLSCATS	GFTFS	DTYALSWMLRQAPGKGLEWVSS	FSG	LDGSTYYADSVKGRFTISRDNSKNTLYLQMN	SLRAEDTAVYYCAK	DRGPVGE-LFDSWGQGLTVTVSS
MEX18_24	EVQLLESGGGLVQPGGSLRLSCVTS	GFTFS	DTYALSWVRQAPGKGLEWVSS	FSG	LDGSTYYTESVKGRFTISRDNSKNTLYLQMN	GLRAEDTAMYYCAK	DRGPVGE-LFDSWGQGLTVTFSS
MEX18_27	EVQLLESGGGLVQPGGSLRLSCATS	GFTFS	TYAMSWVRQAPGKGLEWVSS	FSG	VDDSTYYAESVKGRFTISRDNSKNTLYLQMN	TLRAEDTAVYYCAK	DRGPVGE-LFDSWGQGLTVTVSS
MEX18_36	EVQLLESGGGLVQPGGSLRLTTCATS	GFTFS	SDYAMSWVRQAPGKGLEWVSS	YSG	IDDSTYYADSVKGRFTISRDNSKNTLYLQMN	SLRAEDTAVYYCAK	DRGPVGE-LFDSWGQGLTVTVSS
MEX18_38	EVQLLESGGGLVQPGGSLRLSCAAS	GFTFS	DTYAMGWVRQAPGKGLEWLS	SMTR	TGDNLYADSVKGRFTISRDNSKNTLYLQMN	SLRVEDTAVYYCAK	DRLPVGE-LFDSWGQGLTVTVST
MEX18_41	EVQLLESGGGLVQPGGSLRLSCAAS	GFTFS	SDYAMGWVRQAPQGLDWVSS	CVSG	GGDTYYADSVKGRFTISRDNSKNTVLENNLRPEDTAVYYCAK	QVGE-LFDPHSDHWGQGLTVTVSS	
MEX18_50	EVQLLESGGGLVQPGGSLRLSCVTS	GFTFS	NYGMNWSVRQAPGKGLEWVSG	ITG	SGDDTYADSVKGRFTISRDNSKNTLYLQMN	SLRAEDTAVYYCTK	DR-----LIFDPAHVWGQGLTMVTVSS
MEX18_54	EVQLLESGGGLVQPGGSLRLSCVTS	GFTFS	YAMSWVRQAPGKGLEWVSA	AMTG	SGDDTYADSVKGRFTISRDNSKNTLYLQMN	SLRVEDTAVYYCAK	DRVSGVGE-LQDIWGQGLTVTVSS
MEX18_58	EVQLLESGGGLVQPGGSLRLSCATS	GFTFS	TYAMSWVRQAPGKGLEWVSS	ISG	ADDSTYYAASVKGRFTISRDNSKNTLYLQMN	SLRAEDTAVYYCAK	DRGPVGE-LFDSWGQGLTVTVSS
MEX18_65	EVQLLESGGGLVQPGGSLRLTSCAGS	GFTFS	TYALIWVRQAPGKGLEWVSS	ISY	DSASTYYAESVKGRFTISRDNSKNTLYLQMN	SLRAEDTAVYYCAK	DRITMGVGE-LFPAHVWGQGLTVTVSS
MEX18_79	EVQLLESGGGLVQPGGSLRLSCAAS	GFTFS	RYAMSWVRQAPGKGLEWVSS	ISS	LDGSTYYADSVKGRSAISRDNSKNTLYLQHS	SLRAEDTALYCAK	DRVEKGPVGE-LNASWGQGLTVTVSS
MEX18_80	EVQLLESGGGLVQPGGSLRLTTCATS	GFTFS	SDYAMSWVRQAPGKGLEWVSS	YSG	IDDSTYYADSVKGRFTISRDNSKNTLYLQMN	SLRAEDTAVYYCAK	DRGPVGE-LFDSWGQGLTVTVSS
MEX18_83	EVQLLESGGGLVQPGGSLRLTTCATS	GFTFS	SDYAMSWVRQAPGKGLEWVSS	YSG	IDDSTYYADSVKGRFTISRDNSKNTLYLQMN	SLRAEDTAVYYCAK	DRGPVGE-LFDSWGQGLTVTVSS
MEX18_84	EVQLLESGGGLVQPGGSLRLSCAAS	GFTFS	SYAMNWSVRQAPGKGLEWVSG	IGGRGAIAGDGS	IYYADSVKGRFTISRDNSKNTLYLQMN	GLRVEDTAVYYCAK	DR-----VAFDPGHVWGQGLTVTVSS
MEX18_86	EVQLLESGGGLVQPGGSLRLTTCATS	GFTFS	SDYAMSWVRQAPGKGLEWVSS	YSG	IDDSTYYADSVKGRFTISRDNSKNTLYLQMN	SLRAEDTAVYYCAK	DRGPVGE-LFDSWGQGLTVTVSS
MEX18_89: Z004	EVQLLESGGGLVQPGGSLRLTTCATS	GFTFS	RDYAMSWVRQAPGKGLEWVSS	YSG	IDDSTYYADSVKGRFTISRDNSKNTLYLQMN	SLRAEDTAVYYCAK	DRGPVGE-LFDSWGQGLTVTVSS
MEX18_93	EVQLLESGGGLVQPGGSLRLTTCATS	GFTFS	SDYAMSWVRQAPGKGLEWVSS	YSG	IDDSTYYADSVKGRFTISRDNSKNTLYLQMN	SLRAEDTAVYYCAK	DRGPVGE-LFDSWGQGLTVTVSS
MEX18_94	EVQLLESGGGLVQPGGSLRLTTCATS	GFTFS	SDYAMSWVRQAPGKGLEWVSS	YSG	IDDSTYYADSVKGRFTISRDNSKNTLYLQMN	SLRAEDTAVYYCAK	DRGPVGE-LFDSWGQGLTVTVSS
MEX18 iGL	EVQLLESGGGLVQPGGSLRLSCAAS	GFTFS	SYAMSWVRQAPGKGLEWVSA	ISG	SGGSTYYADSVKGRFTISRDNSKNTLYLQMN	SLRAEDTAVYYCAK	DRGPVGE-LFDTWGQGLTVTVSS

V_L alignment

	1	27	32	50	89	
		CDR1		CDR2		CDR3
IGKV1-5*03	DIQMTQSPSTLSASVGRVITTCRAS	QSIS	SSWLAWYQKPKGAPKLLIY	KASS	LES	GVPSRFSGSGSGTEFTLTISLQPD
IGKJ1*01	-----	-----	-----	-----	-----	QYNSIPNTFGGQTKVEIK
MEX18_07	DIQMTQSPSTLSASVGRVITTCRAS	QSIS	SSWLAWYQKPKGAPKLLIY	KASS	LES	GVPSRFSGSGSGTEFTLTISLQPD
MEX18_15	DIQMTQSPSTLSASVGRVITTCRAS	QINIS	SWLAWYQKPKGAPKLLIY	QAST	LQNGVPSRFSGSGSGTEFTLTISLQPD	FATYYCQHYISVPNTFGGQTKVEIK
MEX18_21	DIQMTQSPSTLSASVGRVITTCRAS	QISIR	WLAWYQKPKGAPKLLIY	KTST	LKSEVPSRFSGSGSGTEFTLTISLQPD	FATYYCQHFHSVPNTFGGQTKVEIK
MEX18_24	DIQMTQSPSTLSASVGRVITTCRAS	QISIK	WLAWYQKPKGAPKLLIY	TTST	LKSGVPSRFSGSGSGTEFTLTISLQPD	FATYYCQHFYSVPNTFGGQTKVEIK
MEX18_27	DIQMTQSPSTLSASVGRVITTCRAS	QISIN	WLAWYQKPKGAPKLLIY	TTST	LKSGVPSRFSGSGSGTEFTLTISLQPD	FATYYCQHFHSVPNTFGGQTKVEIK
MEX18_36	DIQMTQSPSTLSASVGRVITTCRAS	QISIK	WLAWYQKPKGAPKLLIY	TTST	LKSGVPSRFSGSGSGTEFTLTISLQPD	FATYYCQHFYSVPNTFGGQTKVEIK
MEX18_38	DIQMTQSPSTLSAAIGDRVITTCRAS	QISIN	WLAWYQKPKGAPKLLIY	KTST	LKSGVPSRFSGSGSGTEFTLTISLQPD	FATYYCQHYISVPNTFGGQTKVEIK
MEX18_41	DIQMTQSPSTLSVSVGRVITTCRAS	QINIS	SWLAWYQKPKGAPKLLIY	KASS	LER	GVPSRFSGSGSGTEFTLTISLQPD
MEX18_50	DIQMTQSPSTLSASVGRVITTCRAS	QSIS	SSWLAWYQKPKGAPKLLIY	KASS	LES	GVPSRFSGSGSGTEFTLTISLQPD
MEX18_54	DIQMTQSPSTLSASVGRVITTCRAS	QINIS	SWLAWYQKPKGAPKLLIY	KTST	LKSGVPSRFSGSGSGTEFTLTISLQPD	FATYYCQHYISVPNTFGGQTKVEIK
MEX18_58	DIQMTQSPSTLSASVGRVITTCRAS	QISIK	WLAWYQKPKGAPKLLIY	TTST	LKSGVPSRFSGSGSGTEFTLTISLQPD	FATYYCQHFHSVPNTFGGQTKVEIK
MEX18_65	DIQMTQSPSTLSASIGDRVITTCRAS	QSVSG	WLAWYQKPKGAPKLLIY	KASS	TLQSGVPSRFSGSGSGTEFTLTISLQPD	FATYYCQHYISVPNTFGGQTKVEIK
MEX18_79	DIQMTQSPSTLPASVGRVITTCRAS	QINIS	SWLAWYQKPKGAPKLLIY	KASS	ASL	DGVPSRFSGSGSGTEFTLTISLQPD
MEX18_80	DIQMTQSPSTLSASVGRVITTCRAS	QISIK	WLAWYQKPKGAPKLLIY	TTST	LKSGVPSRFSGSGSGTEFTLTISLQPD	FATYYCQHFHSVPNTFGGQTKVEIK
MEX18_83	DIQMTQSPSTLSASVGRVITTCRAS	QSVSK	WLAWYQKPKGAPKLLIY	TTST	LKSGVPSRFSGSGSGTEFTLTISLQPD	FATYYCQHFHSVPNTFGGQTKVEIK
MEX18_84	DIQMTQSPSTLSASVGRVITTCRAS	QSIS	SSWLAWYQKPKGAPKLLIY	KASS	LES	GVPSRFSGSGSGTEFTLTISLQPD
MEX18_86	DIQMTQSPSTLSASVGRVITTCRAS	QISIK	WLAWYQKPKGAPKLLIY	TTST	LKSGVPSRFSGSGSGTEFTLTISLQPD	FATYYCQHFYSVPNTFGGQTKVEIK
MEX18_89: Z004	DIQMTQSPSTLSASVGRVITTCRAS	QISIK	WLAWYQKPKGAPKLLIY	TTST	LKSGVPSRFSGSGSGTEFTLTISLQPD	FATYYCQHFYSVPNTFGGQTKVEIK
MEX18_93	DIQMTQSPSTLSASVGRVITTCRAS	QISIK	WLAWYQKPKGAPKLLIY	TTST	LKSGVPSRFSGSGSGTEFTLTISLQPD	FATYYCQHFYSVPNTFGGQTKVEIK
MEX18_94	DIQMTQSPSTLSASVGRVITTCRAS	QISIK	WLAWYQKPKGAPKLLIY	TTST	LKSGVPSRFSGSGSGTEFTLTISLQPD	FATYYCQHFYSVPNTFGGQTKVEIK
MEX18 iGL	DIQMTQSPSTLSASVGRVITTCRAS	QSIS	SSWLAWYQKPKGAPKLLIY	KASS	LES	GVPSRFSGSGSGTEFTLTISLQPD

Figure S1. Alignments of V_H and V_L sequences of all 20 mature VH3-23/VK1-5 class Abs isolated from donor MEX 18 with their iGL (highlighted)¹. The most common germline gene assignments (top lines) determined by IgBLAST for this set of Abs are shown¹. For V_H, this includes the IGHV3-23*01 V gene segment, the IGHD3-10*01 D gene segment, and the IGHJ4*02 J gene segment. The D gene segment is shown as one possible reading frame. For V_L, this includes the IGKV1-5*03 V gene segment, and the IGKJ1*01 J gene segment. The mature Ab Z004 used for binding studies corresponds to the sequence MEX18_89. The Kabat numbering scheme was used.

V_H alignment

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1          26      33      51      57      93      CDR3
IGHV3-23*01  EVQLLESGGGLVQPQGSRLRLSCAASGPTFSSYAMSHVVRQAPGKGLWVSAISG-SGGST-----YYADSVKGRFTISRDNKNTLYLQMSLRAEDTAVYYCAK-----VLLMFQGE-LI-----
IGHD3-10*01  -----
IGHJ4*02  -----
BRA112_08  EVQLLESGGGLVQPQGSRLRLSCAASGPTFSSYAMSHVVRQAPGKGLWVSSITG-TGGTETT-----YAAASVKGRFTISRDNKNTLYLQMSLRAEDTAVYYCAKDRIGKGLGE-LYSHWGQGLTVTVSS
BRA112_09: Z034  EVQLLESGGGLVQPQGSRLRLSCAASGPTFSSYAMSHVVRQAPGKGLWVSSITG-SGGST-----YAAASVKGRFTISRDNKNTLYLQMSLRAEDTAVYYCAKDRIGKGLGE-LYSHWGQGLTVTVSS
BRA112_21  EVQLLESGGGLVQPQGSRLRLSCAASGPTFSSYAMSHVVRQAPGKGLWVSSITG-RDGST-----YAAASVKGRFTISRDNKNTLYLQMSLRAEDTAVYYCAKDRIGKGLGE-LYSHWGQGLTVTVSS
BRA112_24: Z032  EVQLLESGGGLVQPQGSRLRLSCAASGPTFSSYAMSHVVRQAPGKGLWVSSITG-SGGST-----YAAASVKGRFTISRDNKNTLYLQMSLRAEDTAVYYCAKDRIGKGLGE-LYSHWGQGLTVTVSS
BRA112_33  EVQLLESGGGLVQPQGSRLRLSCAASGPTFSSYAMSHVVRQAPGKGLWVSSITG-SGGST-----YAAASVKGRFTISRDNKNTLYLQMSLRAEDTAVYYCAKDRIGKGLGE-LYSHWGQGLTVTVSS
BRA112_37  EVQLLESGGGLVQPQGSRLRLSCAASGPTFSSYAMSHVVRQAPGKGLWVSSITG-SGGST-----YAAASVKGRFTISRDNKNTLYLQMSLRAEDTAVYYCAKDRIGKGLGE-LYSHWGQGLTVTVSS
BRA112_46: Z031  EVQLLESGGGLVQPQGSRLRLSCAASGPTFSSYAMSHVVRQAPGKGLWVSSITG-SGGST-----YAAASVKGRFTISRDNKNTLYLQMSLRAEDTAVYYCAKDRIGKGLGE-LYSHWGQGLTVTVSS
BRA112_48  EVQLLESGGGLVQPQGSRLRLSCAASGPTFSSYAMSHVVRQAPGKGLWVSSITG-SGGST-----YAAASVKGRFTISRDNKNTLYLQMSLRAEDTAVYYCAKDRIGKGLGE-LYSHWGQGLTVTVSS
BRA112_51  EVQLLESGGGLVQPQGSRLRLSCAASGPTFSSYAMSHVVRQAPGKGLWVSSITG-SGGST-----YAAASVKGRFTISRDNKNTLYLQMSLRAEDTAVYYCAKDRIGKGLGE-LYSHWGQGLTVTVSS
BRA112_56  EVQLLESGGGLVQPQGSRLRLSCAASGPTFSSYAMSHVVRQAPGKGLWVSSITG-SGGST-----YAAASVKGRFTISRDNKNTLYLQMSLRAEDTAVYYCAKDRIGKGLGE-LYSHWGQGLTVTVSS
BRA112_65  EVQLLESGGGLVQPQGSRLRLSCAASGPTFSSYAMSHVVRQAPGKGLWVSSITG-SGGST-----YAAASVKGRFTISRDNKNTLYLQMSLRAEDTAVYYCAKDRIGKGLGE-LYSHWGQGLTVTVSS
BRA112_69  EVQLLESGGGLVQPQGSRLRLSCAASGPTFSSYAMSHVVRQAPGKGLWVSSITG-SGGST-----YAAASVKGRFTISRDNKNTLYLQMSLRAEDTAVYYCAKDRIGKGLGE-LYSHWGQGLTVTVSS
BRA112_71: Z035  EVQLLESGGGLVQPQGSRLRLSCAASGPTFSSYAMSHVVRQAPGKGLWVSSITG-SGGST-----YAAASVKGRFTISRDNKNTLYLQMSLRAEDTAVYYCAKDRIGKGLGE-LYSHWGQGLTVTVSS
BRA112_91: Z036  EVQLLESGGGLVQPQGSRLRLSCAASGPTFSSYAMSHVVRQAPGKGLWVSSITG-SGGST-----YAAASVKGRFTISRDNKNTLYLQMSLRAEDTAVYYCAKDRIGKGLGE-LYSHWGQGLTVTVSS
BRA112_94  EVQLLESGGGLVQPQGSRLRLSCAASGPTFSSYAMSHVVRQAPGKGLWVSSITG-SGGST-----YAAASVKGRFTISRDNKNTLYLQMSLRAEDTAVYYCAKDRIGKGLGE-LYSHWGQGLTVTVSS
BRA112_IGL  EVQLLESGGGLVQPQGSRLRLSCAASGPTFSSYAMSHVVRQAPGKGLWVSSITG-SGGST-----YAAASVKGRFTISRDNKNTLYLQMSLRAEDTAVYYCAKDRIGKGLGE-LYSHWGQGLTVTVSS

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V_L alignment

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1          27      32      50      59      CDR3
IGKV1-5*03  DIQMTQSPSTLSASVDGRVITTCRASGISISHLAWYQQKPGKAPKLLIYKASLESQVPSRFSGSGSGTEFTLTISLQDDFATYYCQYNSYS-----
IGKJ1*01  -----
BRA112_08  DIQMTQSPSTLSASVDGRVITTCRASGISISHLAWYQQKPGKAPKLLIYKASLESQVPSRFSGSGSGTEFTLTISLQDDFATYYCQYNSYS-----
BRA112_09: Z034  DIQMTQSPSTLSASVDGRVITTCRASGISISHLAWYQQKPGKAPKLLIYKASLESQVPSRFSGSGSGTEFTLTISLQDDFATYYCQYNSYS-----
BRA112_21  DIQMTQSPSTLSASVDGRVITTCRASGISISHLAWYQQKPGKAPKLLIYKASLESQVPSRFSGSGSGTEFTLTISLQDDFATYYCQYNSYS-----
BRA112_24: Z032  DIQMTQSPSTLSASVDGRVITTCRASGISISHLAWYQQKPGKAPKLLIYKASLESQVPSRFSGSGSGTEFTLTISLQDDFATYYCQYNSYS-----
BRA112_33  DIQMTQSPSTLSASVDGRVITTCRASGISISHLAWYQQKPGKAPKLLIYKASLESQVPSRFSGSGSGTEFTLTISLQDDFATYYCQYNSYS-----
BRA112_37  DIQMTQSPSTLSASVDGRVITTCRASGISISHLAWYQQKPGKAPKLLIYKASLESQVPSRFSGSGSGTEFTLTISLQDDFATYYCQYNSYS-----
BRA112_46: Z031  DIQMTQSPSTLSASVDGRVITTCRASGISISHLAWYQQKPGKAPKLLIYKASLESQVPSRFSGSGSGTEFTLTISLQDDFATYYCQYNSYS-----
BRA112_48  DIQMTQSPSTLSASVDGRVITTCRASGISISHLAWYQQKPGKAPKLLIYKASLESQVPSRFSGSGSGTEFTLTISLQDDFATYYCQYNSYS-----
BRA112_51  DIQMTQSPSTLSASVDGRVITTCRASGISISHLAWYQQKPGKAPKLLIYKASLESQVPSRFSGSGSGTEFTLTISLQDDFATYYCQYNSYS-----
BRA112_56  DIQMTQSPSTLSASVDGRVITTCRASGISISHLAWYQQKPGKAPKLLIYKASLESQVPSRFSGSGSGTEFTLTISLQDDFATYYCQYNSYS-----
BRA112_65  DIQMTQSPSTLSASVDGRVITTCRASGISISHLAWYQQKPGKAPKLLIYKASLESQVPSRFSGSGSGTEFTLTISLQDDFATYYCQYNSYS-----
BRA112_69  DIQMTQSPSTLSASVDGRVITTCRASGISISHLAWYQQKPGKAPKLLIYKASLESQVPSRFSGSGSGTEFTLTISLQDDFATYYCQYNSYS-----
BRA112_71: Z035  DIQMTQSPSTLSASVDGRVITTCRASGISISHLAWYQQKPGKAPKLLIYKASLESQVPSRFSGSGSGTEFTLTISLQDDFATYYCQYNSYS-----
BRA112_91: Z036  DIQMTQSPSTLSASVDGRVITTCRASGISISHLAWYQQKPGKAPKLLIYKASLESQVPSRFSGSGSGTEFTLTISLQDDFATYYCQYNSYS-----
BRA112_94  DIQMTQSPSTLSASVDGRVITTCRASGISISHLAWYQQKPGKAPKLLIYKASLESQVPSRFSGSGSGTEFTLTISLQDDFATYYCQYNSYS-----
BRA112_IGL  DIQMTQSPSTLSASVDGRVITTCRASGISISHLAWYQQKPGKAPKLLIYKASLESQVPSRFSGSGSGTEFTLTISLQDDFATYYCQYNSYS-----

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Figure S2. Alignments of V_H and V_L sequences of all 15 mature VH3-23/VK1-5 class Abs isolated from donor BRA 112 with their iGL (highlighted)¹. The most common germline gene assignments (top lines) determined by IgBLAST for this set of Abs are shown¹. For V_H, this includes the IGHV3-23*01 V gene, the IGHD3-10*01 D gene, and the IGHJ4*02 J gene. The D gene segment is shown as one possible reading frame. For V_L, this includes the IGKV1-5*03 V gene and IGKJ1*01 J gene. The Z03X mature Abs (Z034, Z032, Z031, Z035, and Z036) used for binding studies correspond to the sequences BRA112_09, BRA112_24, BRA112_46, BRA112_71, and BRA112_91 respectively. The Kabat numbering scheme was used.

V_H alignment

	1	26	33	51	57	93	
		CDR1		CDR2		CDR3	
IGHV3-23*03	EVQLLESGGGLVPGGSLRLSCAAS	GFTFSSYAMSWVRQAPGKGLEWVS	IYSGGSS	TYADSVKGRFTISRDN	SNTLYLQMN	SLRAEDTAVIYCAK	-----
IGHD6-19*01	-----	-----	-----	-----	-----	-----	-----GYSSGWY-----
IGHJ4*02	-----	-----	-----	-----	-----	-----	-----YFDVWGQGLVTVSS-----
MEX105_01	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMAWVRQAPGKGLEWVS	IYNGDDST	TYAKSVKGRFTISRDS	SQNTLSLQMN	SLRAEDTAVIYCVKDR	-----DTGWSSIVDWGQGLVTVSS-----
MEX105_02	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMAWVRQAPGKGLEWVS	IYNGDDST	TYAKSVKGRFTISRDS	SQNTLSLQMN	SLRAEDTAVIYCVKDR	-----DTGWSSIVDWGQGLVTVSS-----
MEX105_04	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMAWVRQAPGKGLEWVS	IYNGDDST	TYAKSVKGRFTISRDS	SQNTLSLQMN	SLRAEDTAVIYCVKDR	-----DTGWSSIVDWGQGLVTVSS-----
MEX105_05	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMAWVRQAPGKGLEWVS	IYNGDDST	TYAKSVKGRFTISRDS	SQNTLSLQMN	SLRAEDTAVIYCVKDR	-----DTGWSSIVDWGQGLVTVSS-----
MEX105_09	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMAWVRQAPGKGLEWVS	IYNGDDST	TYAKSVKGRFTISRDS	SQNTLSLQMN	SLRAEDTAVIYCVKDR	-----DTGWSSIVDWGQGLVTVSS-----
MEX105_11	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMAWVRQAPGKGLEWVS	IYNGDDST	TYAKSVKGRFTISRDS	SQNTLSLQMN	SLRAEDTAVIYCVKDR	-----DTGWSSIVDWGQGLVTVSS-----
MEX105_14	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMAWVRQAPGKGLEWVS	IYNGDDST	TYAKSVKGRFTISRDS	SQNTLSLQMN	SLRAEDTAVIYCVKDR	-----DTGWSSIVDWGQGLVTVSS-----
MEX105_15	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMSWVRQAPGKGLEWVS	ISAREDS	TYAASVGRFTISRDS	SNTLYLQMN	SLRAEDTAVIYCAKDR	-----LQGVGE-LYESWGQGLVTVSS-----
MEX105_23	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMAWVRQAPGKGLEWVS	IYNGDDST	TYAKSVKGRFTISRDS	SQNTLSLQMN	SLRAEDTAVIYCVKDR	-----DTGWSSIVDWGQGLVTVSS-----
MEX105_25	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMAWVRQAPGKGLEWVS	IYNGDDST	TYAKSVKGRFTISRDS	SQNTLSLQMN	SLRAEDTAVIYCVKDR	-----DTGWSSIVDWGQGLVTVSS-----
MEX105_27	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMAWVRQAPGKGLEWVS	IYNGDDST	TYAKSVKGRFTISRDS	SQNTLSLQMN	SLRAEDTAVIYCVKDR	-----DTGWSSIVDWGQGLVTVSS-----
MEX105_28	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMAWVRQAPGKGLEWVS	IYNGDDST	TYAKSVKGRFTISRDS	SQNTLSLQMN	SLRAEDTAVIYCVKDR	-----DTGWSSIVDWGQGLVTVSS-----
MEX105_33	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMAWVRQAPGKGLEWVS	IYNGDDST	TYAKSVKGRFTISRDS	SQNTLSLQMN	SLRAEDTAVIYCVKDR	-----DTGWSSIVDWGQGLVTVSS-----
MEX105_37	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMAWVRQAPGKGLEWVS	IYNGDDST	TYAKSVKGRFTISRDS	SQNTLSLQMN	SLRAEDTAVIYCVKDR	-----DTGWSSIVDWGQGLVTVSS-----
MEX105_39	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMSWVRQAPGKGLEWVS	ISAREDS	TYAASVGRFTISRDS	SNTLYLQMN	SLRAEDTAVIYCAKDR	-----LIEFVGQGLVTVSS-----
MEX105_42: Z006	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMAWVRQAPGKGLEWVS	IYNGDDST	TYAKSVKGRFTISRDS	SQNTLSLQMN	SLRAEDTAVIYCVKDR	-----DTGWSSIVDWGQGLVTVSS-----
MEX105_45	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMAWVRQAPGKGLEWVS	IYNGDDST	TYAKSVKGRFTISRDS	SQNTLSLQMN	SLRAEDTAVIYCVKDR	-----DTGWSSIVDWGQGLVTVSS-----
MEX105_48	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMAWVRQAPGKGLEWVS	IYNGDDST	TYAKSVKGRFTISRDS	SQNTLSLQMN	SLRAEDTAVIYCVKDR	-----DTGWSSIVDWGQGLVTVSS-----
MEX105_50	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMAWVRQAPGKGLEWVS	IYNGDDST	TYAKSVKGRFTISRDS	SQNTLSLQMN	SLRAEDTAVIYCVKDR	-----DTGWSSIVDWGQGLVTVSS-----
MEX105_51	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMAWVRQAPGKGLEWVS	IYNGDDST	TYAKSVKGRFTISRDS	SQNTLSLQMN	SLRAEDTAVIYCVKDR	-----DTGWSSIVDWGQGLVTVSS-----
MEX105_54	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMAWVRQAPGKGLEWVS	IYNGDDST	TYAKSVKGRFTISRDS	SQNTLSLQMN	SLRAEDTAVIYCVKDR	-----DTGWSSIVDWGQGLVTVSS-----
MEX105_60	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMAWVRQAPGKGLEWVS	IYNGDDST	TYAKSVKGRFTISRDS	SQNTLSLQMN	SLRAEDTAVIYCVKDR	-----DTGWSSIVDWGQGLVTVSS-----
MEX105_64	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMAWVRQAPGKGLEWVS	IYNGDDST	TYAKSVKGRFTISRDS	SQNTLSLQMN	SLRAEDTAVIYCVKDR	-----DTGWSSIVDWGQGLVTVSS-----
MEX105_66	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMAWVRQAPGKGLEWVS	IYNGDDST	TYAKSVKGRFTISRDS	SQNTLSLQMN	SLRAEDTAVIYCVKDR	-----DTGWSSIVDWGQGLVTVSS-----
MEX105_78	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMAWVRQAPGKGLEWVS	IYNGDDST	TYAKSVKGRFTISRDS	SQNTLSLQMN	SLRAEDTAVIYCVKDR	-----DTGWSSIVDWGQGLVTVSS-----
MEX105_87	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMAWVRQAPGKGLEWVS	IYNGDDST	TYAKSVKGRFTISRDS	SQNTLSLQMN	SLRAEDTAVIYCVKDR	-----DTGWSSIVDWGQGLVTVSS-----
MEX105_88	EVQLLESGGGLVPGGSLRLSCAAS	GFTFRRYAMAWVRQAPGKGLEWVS	IYNGDDST	TYADSVKGRFTISRDN	SNTLYLQMN	SLRAEDTAVIYCAKDR	-----DTGWSSIVDWGQGLVTVSS-----

V_L alignment

	1	27	32	50	89	
		CDR1		CDR2		CDR3
IGKV1-5*03	DIQMTQSPSTLSASVGRVTITCRAS	QSISNW	LAWYQKPKGAPKLLIY	KAS	SLESGVPSRFSGSGSGTEFL	TISSLQPD
IGKJ1*01	-----	-----	-----	-----	-----	-----WTFGQGTKEIK-----
MEX105_1	DIQMTQSPSTLSASVGRVTITCRAS	QTIAMN	LAWYQKPKGAPKLLIY	QAS	ILESGVPSRFSGSGSGTEFL	TISSLQPD
MEX105_2	DIQMTQSPSTLSASVGRVTITCRAS	QTLGNW	LAWYQKPKGAPKLLIY	QAS	ILESGVPSRFSGSGSGTEFL	TISSLQPD
MEX105_4	DIQMTQSPSTLSASVGRVTITCRAS	QTIISW	LAWYQKPKGAPKLLIY	QAS	ILESGVPSRFSGSGSGTEFL	TISSLQPD
MEX105_5	DIQMTQSPSTLSASVGRVTITCRAS	QTIISW	LAWYQKPKGAPKLLIY	QAS	ILESGVPSRFSGSGSGTEFL	TISSLQPD
MEX105_9	DIQMTQSPSTLSASVGRVTITCRAS	HTIGNW	LAWYQKPKGAPKLLIY	QAS	ILESGVPSRFSGSGSGTEFL	TISSLQPD
MEX105_11	DIQMTQSPSTLSASVGRVTITCRAS	QTIISW	LAWYQKPKGAPKLLIY	QAS	ILESGVPSRFSGSGSGTEFL	TISSLQPD
MEX105_14	DIQMTQSPSTLSASVGRVTITCRAS	RSISNW	LAWYQKPKGAPKLLIY	TAS	LETGVPSRFSGSGSGTEFL	TISSLQPD
MEX105_15	DIQMTQSPSTLSASVGRVTITCRAS	QNIINW	LAWYQKPKGAPKLLIY	MAS	LSQGVPSRFSGSGSGTEFL	TISSLQPD
MEX105_23	DIQMTQSPSTLSASVGRVTITCRAS	QNVDNW	LAWYQKPKGAPKLLIY	QAS	ILESGVPSRFSGSGSGTEFL	TISSLQPD
MEX105_25	DIQMTQSPSTLSASVGRVTITCRAS	QTIISW	LAWYQKPKGAPKLLIY	QAS	ILESGVPSRFSGSGSGTEFL	TISSLQPD
MEX105_27	DIQMTQSPSTLSASVGRVTITCRAS	RTIGNW	LAWYQKPKGAPKLLIY	QAS	ILESGVPSRFSGSGSGTEFL	TISSLQPD
MEX105_33	DIQMTQSPSTLSASVGRVTITCRAS	QTIISW	LAWYQKPKGAPKLLIY	QAS	ILESGVPSRFSGSGSGTEFL	TISSLQPD
MEX105_37	DIQMTQSPSTLSASVGRVTITCRAS	QTIISW	LAWYQKPKGAPKLLIY	QAS	ILESGVPSRFSGSGSGTEFL	TISSLQPD
MEX105_39	DIQMTQSPSTLSASVGRVTITCRAS	QNIINW	LAWYQKPKGAPKLLIY	KAS	LSQGVPSRFSGSGSGTEFL	TISSLQPD
MEX105_42: Z006	DIQMTQSPSTLSASVGRVTITCRAS	QTIISW	LAWYQKPKGAPKLLIY	QAS	ILESGVPSRFSGSGSGTEFL	TISSLQPD
MEX105_45	DIQMTQSPSTLSASVGRVTITCRAS	QTIISW	LAWYQKPKGAPKLLIY	QAS	ILESGVPSRFSGSGSGTEFL	TISSLQPD
MEX105_48	DIQMTQSPSTLSASVGRVTITCRAS	QTIISW	LAWYQKPKGAPKLLIY	QAS	ILESGVPSRFSGSGSGTEFL	TISSLQPD
MEX105_50	DIQMTQSPSTLSASVGRVTITCRAS	HSISW	LAWYQKPKGAPKLLIY	QAS	ILESGVPSRFSGSGSGTEFL	TISSLQPD
MEX105_51	DIQMTQSPSTLSASVGRVTITCRAS	QTIISW	LAWYQKPKGAPKLLIY	QAS	ILESGVPSRFSGSGSGTEFL	TISSLQPD
MEX105_54	DIQMTQSPSTLSASVGRVTITCRAS	RTIGNW	LAWYQKPKGAPKLLIY	QAS	ILESGVPSRFSGSGSGTEFL	TISSLQPD
MEX105_60	DIQMTQSPSTLSASVGRVTITCRAS	QTIISW	LAWYQKPKGAPKLLIY	QAS	ILESGVPSRFSGSGSGTEFL	TISSLQPD
MEX105_64	DIQMTQSPSTLSASVGRVTITCRAS	RTIGNW	LAWYQKPKGAPKLLIY	QAS	ILESGVPSRFSGSGSGTEFL	TISSLQPD
MEX105_66	DIQMTQSPSTLSASVGRVTITCRAS	QTIISW	LAWYQKPKGAPKLLIY	QAS	ILESGVPSRFSGSGSGTEFL	TISSLQPD
MEX105_78	DIQMTQSPSTLSASVGRVTITCRAS	HTIGNW	LAWYQKPKGAPKLLIY	QAS	ILESGVPSRFSGSGSGTEFL	TISSLQPD
MEX105_87	DIQMTQSPSTLSASVGRVTITCRAS	HTISNW	LAWYQKPKGAPKLLIY	QAS	ILESGVPSRFSGSGSGTEFL	TISSLQPD
MEX105_88	DIQMTQSPSTLSASVGRVTITCRAS	QTIISW	LAWYQKPKGAPKLLIY	QAS	ILESGVPSRFSGSGSGTEFL	TISSLQPD

Figure S3. Alignments of V_H and V_L sequences of all 27 mature VH3-23/VK1-5 class Abs isolated from donor MEX 105 with CDRs highlighted¹. The mature Ab Z006 used for binding studies corresponds to the sequence MEX105_42. The most common germline gene assignments (top lines) determined by IgBLAST for this set of Abs are shown¹. For V_H, this includes the IGHV3-23*03 V gene segment, the IGHJ4*02 J gene segment, and the IGHJ4*02 J gene segment. The D gene segment is shown as one possible reading frame. For V_L, this includes the IGKV1-5*03 V gene segment and the IGKJ1*01 J gene segment. The Kabat numbering scheme was used.

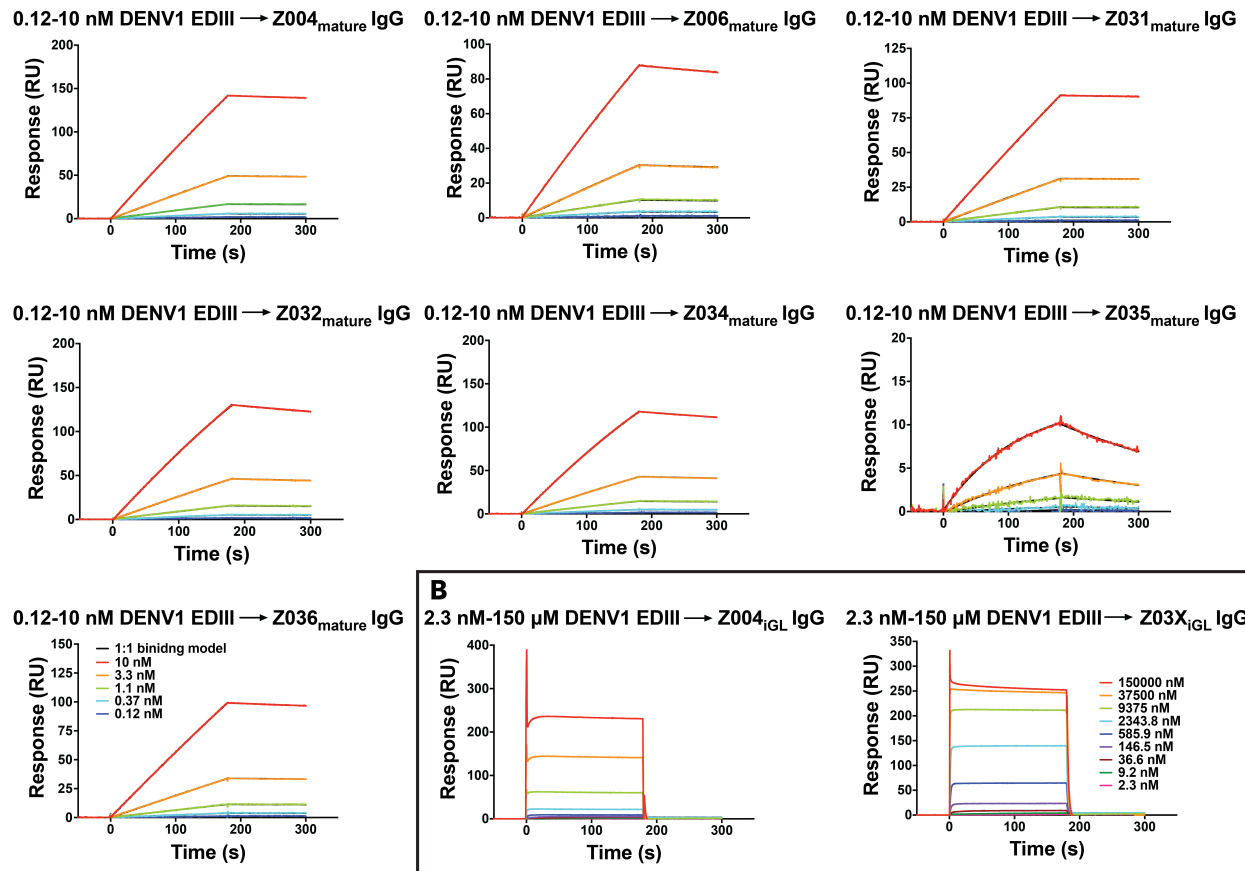
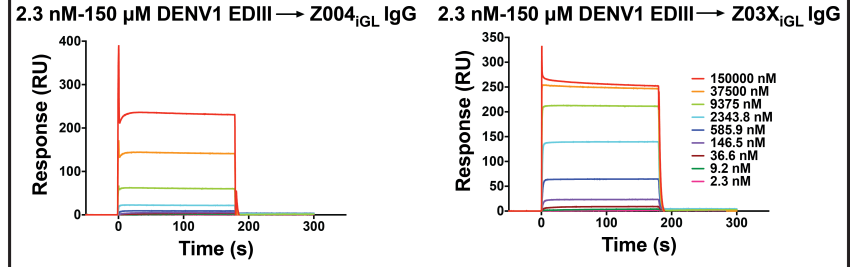
A**B**

Figure S4. SPR binding assays with DENV1 EDIII. IgGs were captured on a protein A biosensor chip, and the indicated concentrations of DENV1 EDIII were injected. Sensorgrams are indicated in colors representing different injected concentrations. **A.** Mature IgGs binding to DENV1 EDIII. Fits to a 1:1 binding model are in black; since the models very closely fit the data, the models are only slightly visible. Residual plots for the 1:1 binding model fitting are shown in SI Appendix, Figure S7A. Two independent experiments were performed; the other set of sensorgrams is shown in Si Appendix, Figure S6. **B.** iGL IgGs binding to DENV1 EDIII. Fitting curves for equilibrium binding responses are shown in Figure 3. Y-axes show response units (RU).

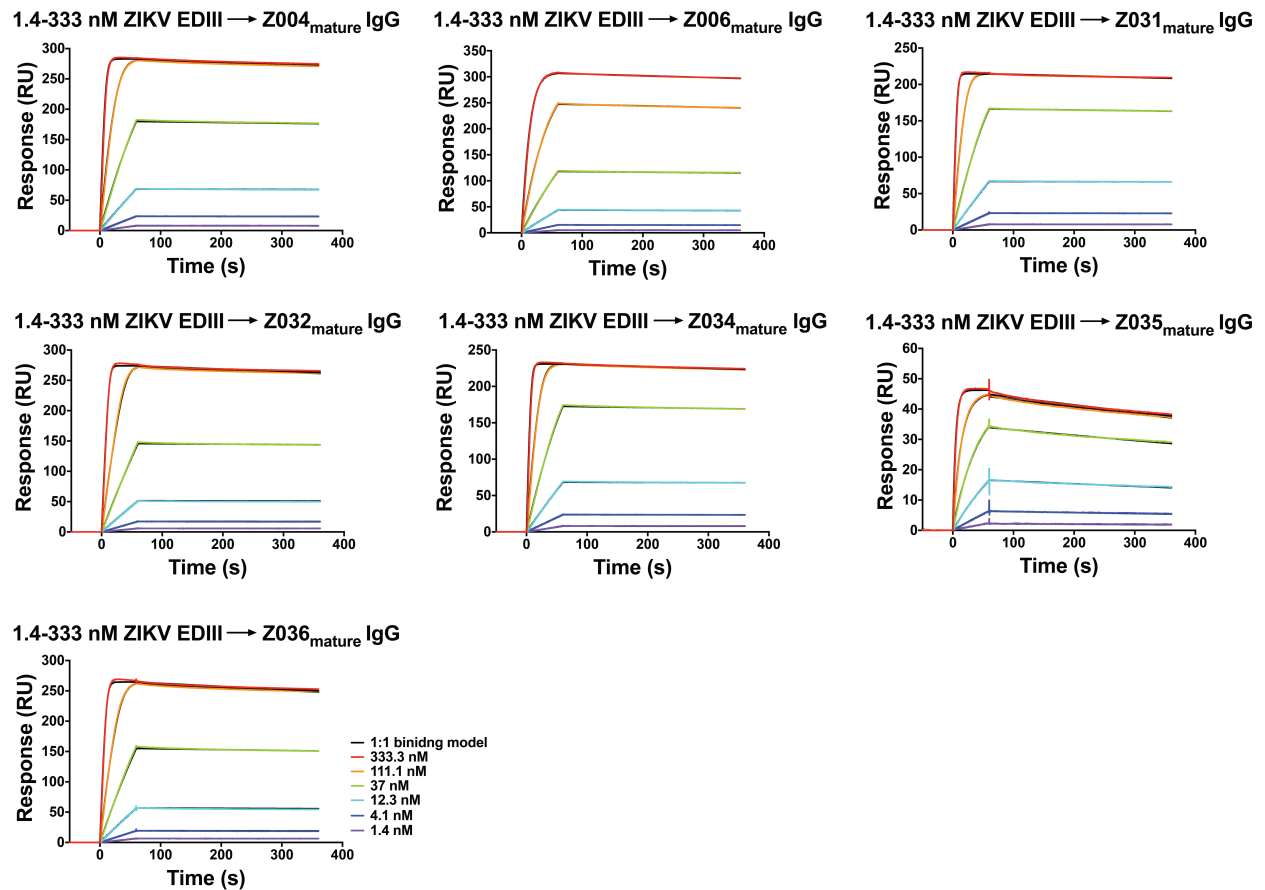


Figure S5. SPR binding assays with ZIKV EDIII. IgGs were captured on a protein A biosensor chip, and the indicated concentrations of ZIKV EDIII were injected. Sensorgrams are indicated in colors representing different injected concentrations. Mature IgGs binding to ZIKV EDIII. Fits to a 1:1 binding model are in black; since the models very closely fit the data, the models are only slightly visible. Residual plots for the 1:1 binding model fitting are shown in SI Appendix, Figure S7B. Y-axes show response units (RU). Two independent experiments were performed; the other set of sensorgrams is shown in Figure 2A.

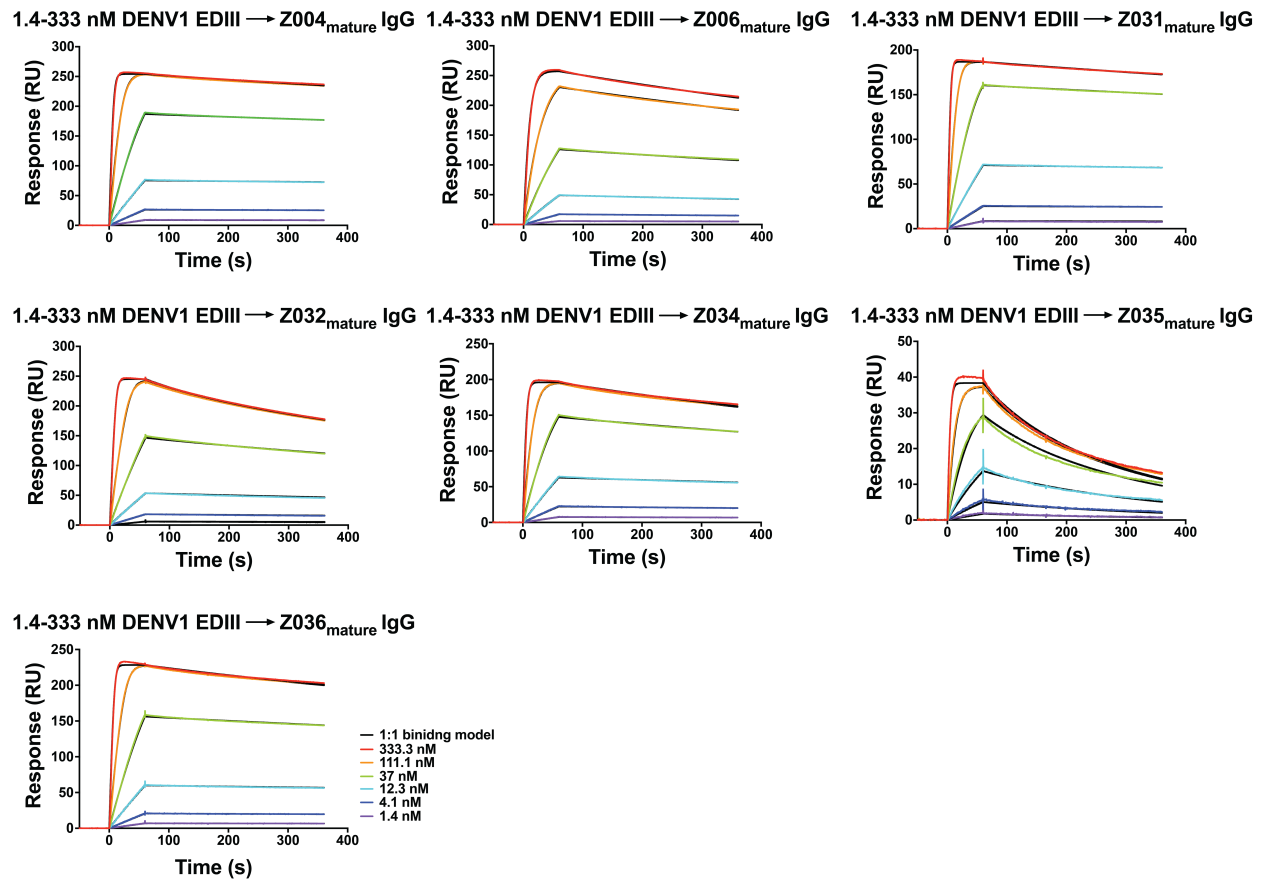


Figure S6. SPR binding assays with DENV1 EDIII. IgGs were captured on a protein A biosensor chip, and the indicated concentrations of DENV1 EDIII were injected. Sensorgrams are indicated in colors representing different injected concentrations. Mature IgGs binding to DENV1 EDIII. Fits to a 1:1 binding model are in black; since the models very closely fit the data, the models are only slightly visible. Residual plots for the 1:1 binding model fitting are shown in SI Appendix, Figure S7B. Y-axes show response units (RU). Two independent experiments were performed; the other set of sensorgrams is shown in SI Appendix, Figure S4A.

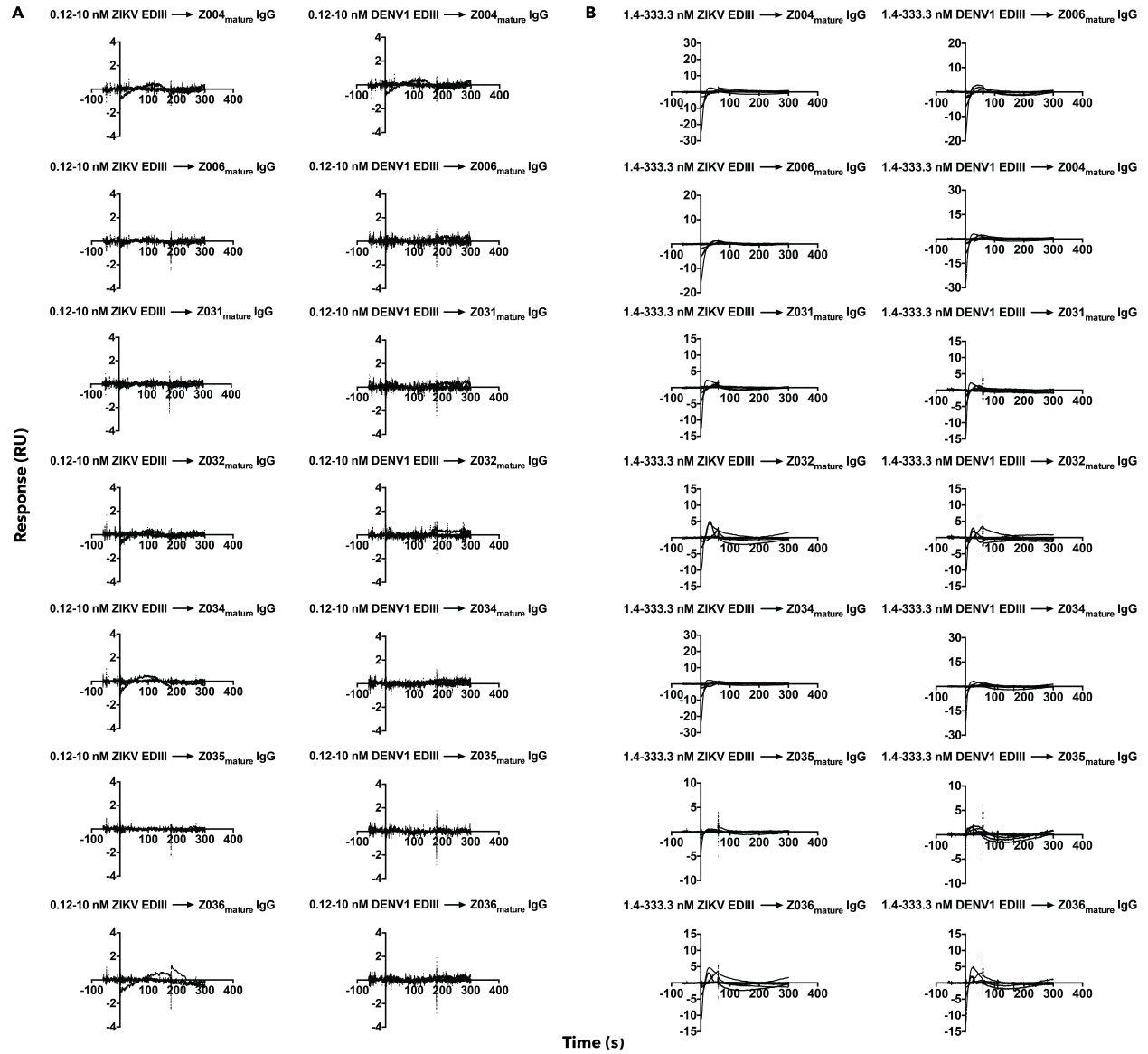


Figure S7. Residual plots for binding model fitting to SPR sensorgrams of ZIKV EDIII and DENV1 EDIII binding to mature IgGs from two independent experiments. **A.** Related to Figure 2A and SI Appendix, FigureS4A. **B.** Related to SI Appendix, Figures S5-S6. Y-axes shows response units (RU).

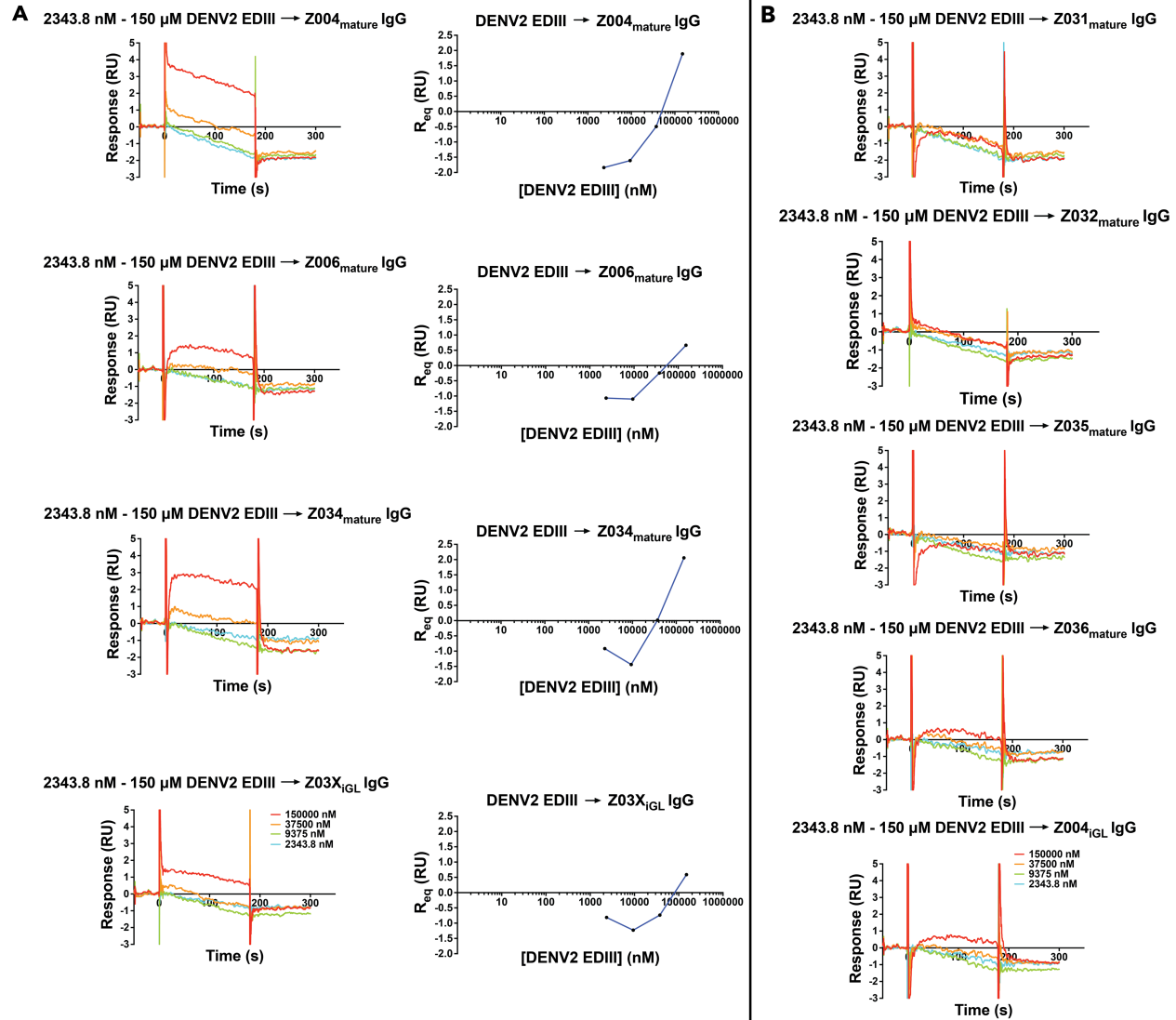


Figure S8. SPR binding assays for IgGs (ligands) interacting with DENV2 EDIII (analyte). IgGs were captured on a protein A biosensor chip, and the indicated concentrations of DENV2 EDIII were injected. Sensorgrams are indicated in colors representing different injected concentrations. **A.** Three mature IgG and one iGL IgG binding to DENV2 EDIII. Sensorgrams (left) and equilibrium binding curves (right) demonstrate weak binding of DENV2 EDIII to Z004_{mature}, Z006_{mature}, Z034_{mature}, and Z03X_{iGL} IgGs. **B.** Four mature IgGs and one iGL IgG showed no binding to DENV2 EDIII. No detectable binding was found for Z031_{mature}, Z032_{mature}, Z035_{mature}, Z036_{mature}, and Z004_{iGL} at concentrations ≤ 150 μ M. Negative values indicate more DENV2 EDIII bound to the reference flow cell, HIV-1 IgG N6, than to the mature and iGL anti-ZIKV IgGs. Y-axes show response units (RU). Four independent injections were performed.

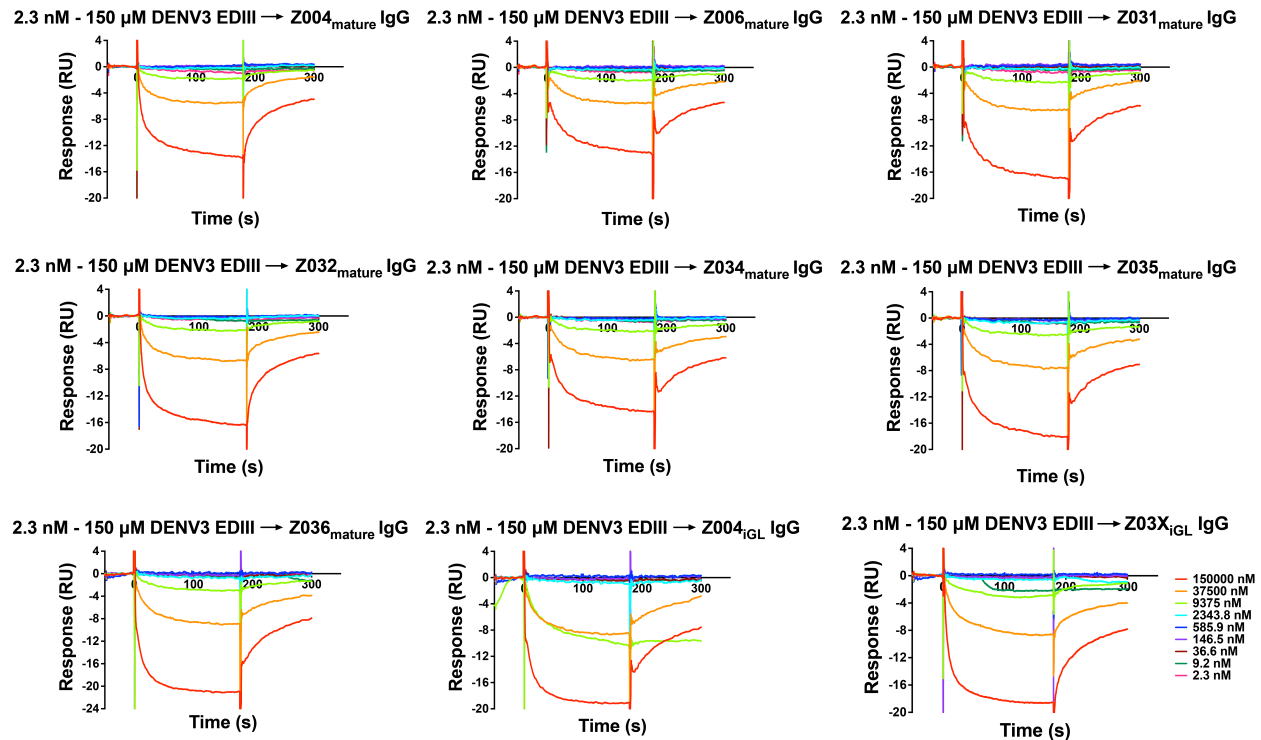


Figure S9. SPR binding assays for IgGs (ligands) interacting with DENV3 EDIII (analyte). IgGs were captured on a protein A biosensor chip, and the indicated concentrations of DENV3 EDIII were injected. Sensorgrams are indicated in colors representing different injected concentrations. No detectable binding was found for Z004_{mature}, Z006_{mature}, Z031_{mature}, Z032_{mature}, Z034_{mature}, Z035_{mature}, Z036_{mature}, Z004_{iGL}, and Z03X_{iGL} at concentrations $\leq 150 \mu\text{M}$. Negative values indicate more DENV3 EDIII bound to the reference flow cell, HIV-1 IgG N6, than to the mature and iGL anti-ZIKV IgGs. Y-axes show response units (RU). Nine independent injections were performed.

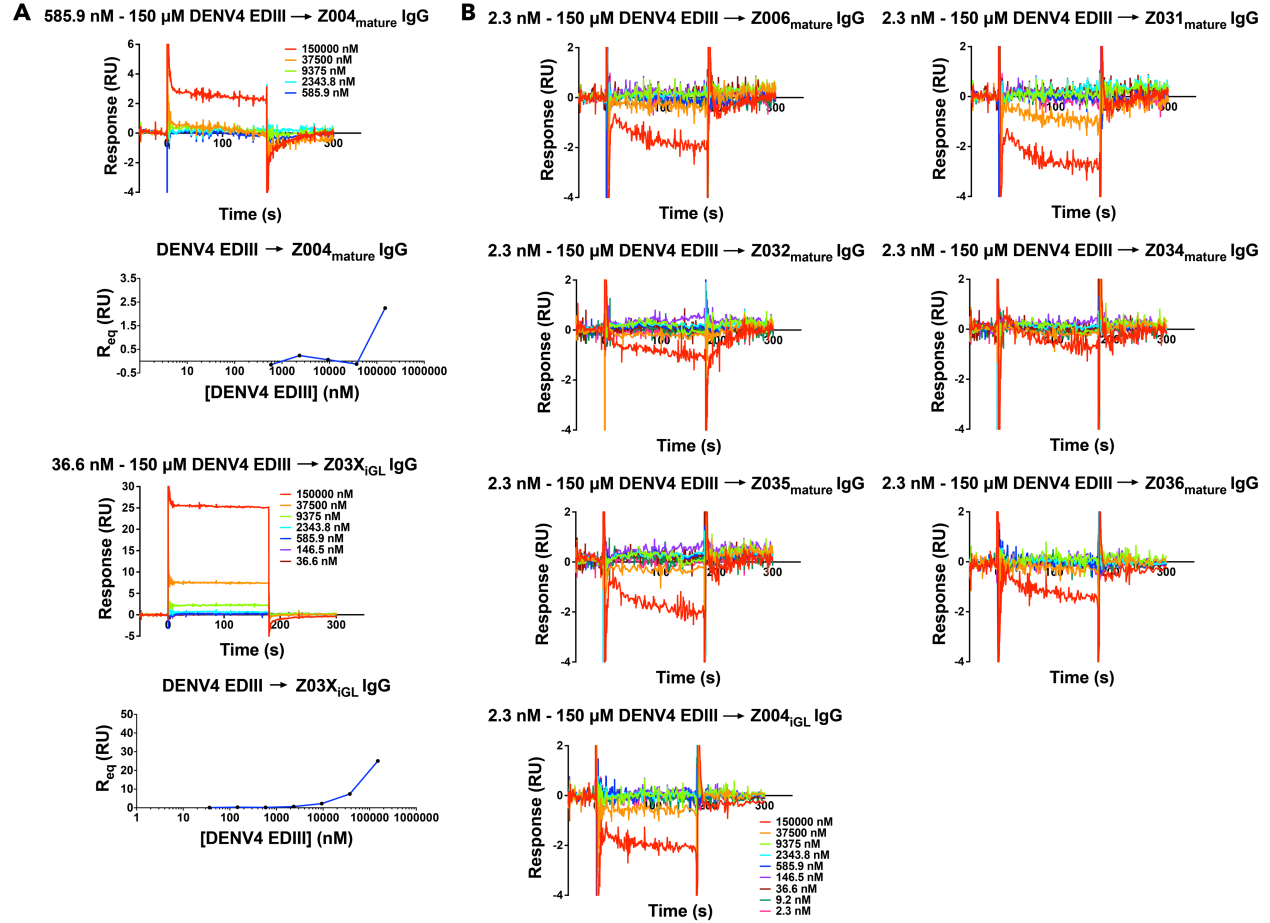


Figure S10. SPR binding assays for IgGs (ligands) interacting with DENV4 EDIII (analyte). IgGs were captured on a protein A biosensor chip, and the indicated concentrations of DENV4 EDIII were injected. Sensorgrams are indicated in colors representing different injected concentrations. **A.** One mature IgG and one iGL IgG binding to DENV4 EDIII. Sensorgrams (left) and equilibrium binding curves (right) demonstrate weak binding of DENV4 EDIII to Z004_{mature} and Z03X_{iGL} IgGs. **B.** Six mature IgGs and one iGL IgG showed no binding DENV4 EDIII. No detectable binding was found for Z004_{mature}, Z006_{mature}, Z031_{mature}, Z032_{mature}, Z034_{mature}, Z035_{mature}, Z036_{mature}, and Z004_{iGL} at concentrations ≤ 150 μ M. Negative values indicate more DENV4 EDIII bound to the reference flow cell, HIV-1 IgG N6, than to the mature and iGL anti-ZIKV IgGs. Y-axes show response units (RU). Five to nine independent injections were performed.

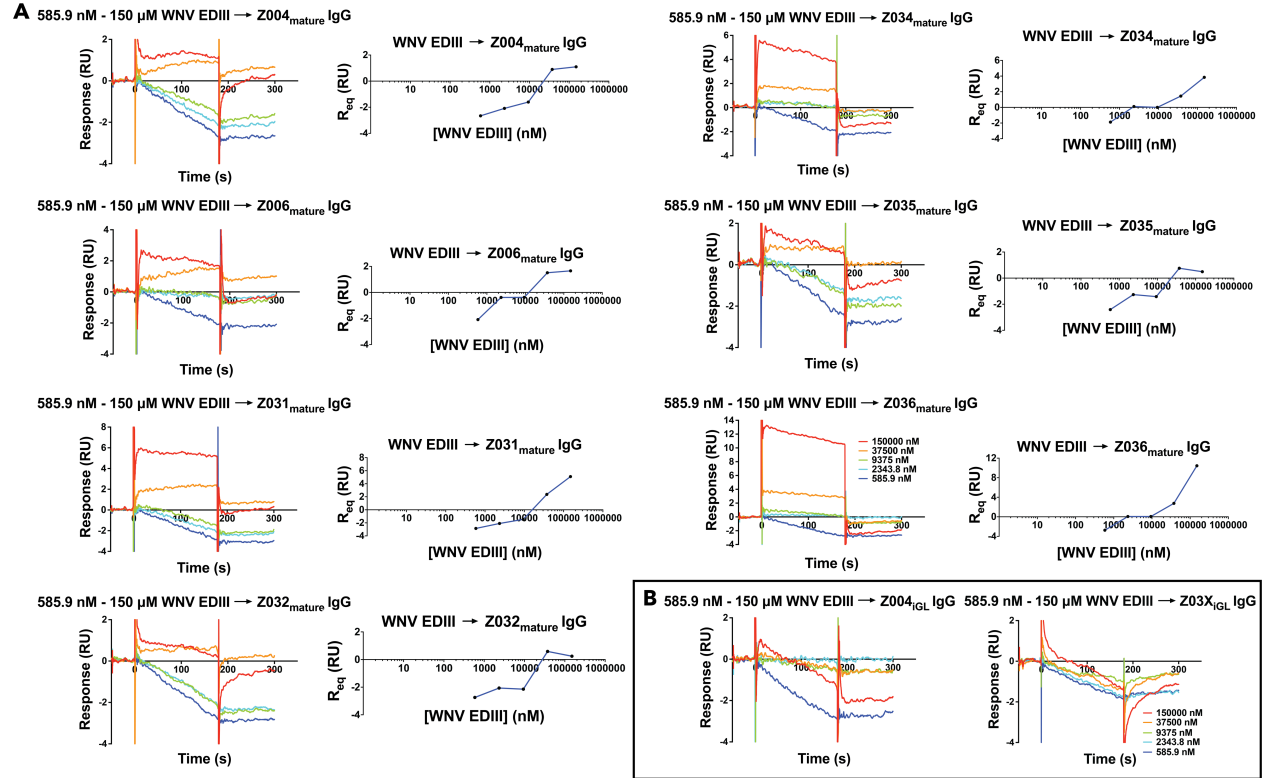


Figure S11. SPR binding assays for IgGs (ligands) interacting with WNV EDIII (analyte). IgGs were captured on a protein A biosensor chip, and the indicated concentrations of WNV EDIII were injected. Sensorgrams are indicated in colors representing different injected concentrations. **A.** Mature IgGs binding to WNV EDIII. Sensorgrams (left) and equilibrium binding curves (right) demonstrate weak binding of WNV EDIII to Z004_{mature}, Z006_{mature}, Z031_{mature}, Z032_{mature}, Z034_{mature}, Z035_{mature}, and Z036_{mature}. **B.** No detectable binding was found for Z004_{iGL} and Z03X_{iGL} at concentrations $\leq 150 \mu$ M. Negative values indicate more WNV EDIII bound to the reference flow cell, HIV-1 IgG N6, than to the mature and iGL anti-ZIKV IgGs. Y-axes show response units (RU). Five independent injections were performed.

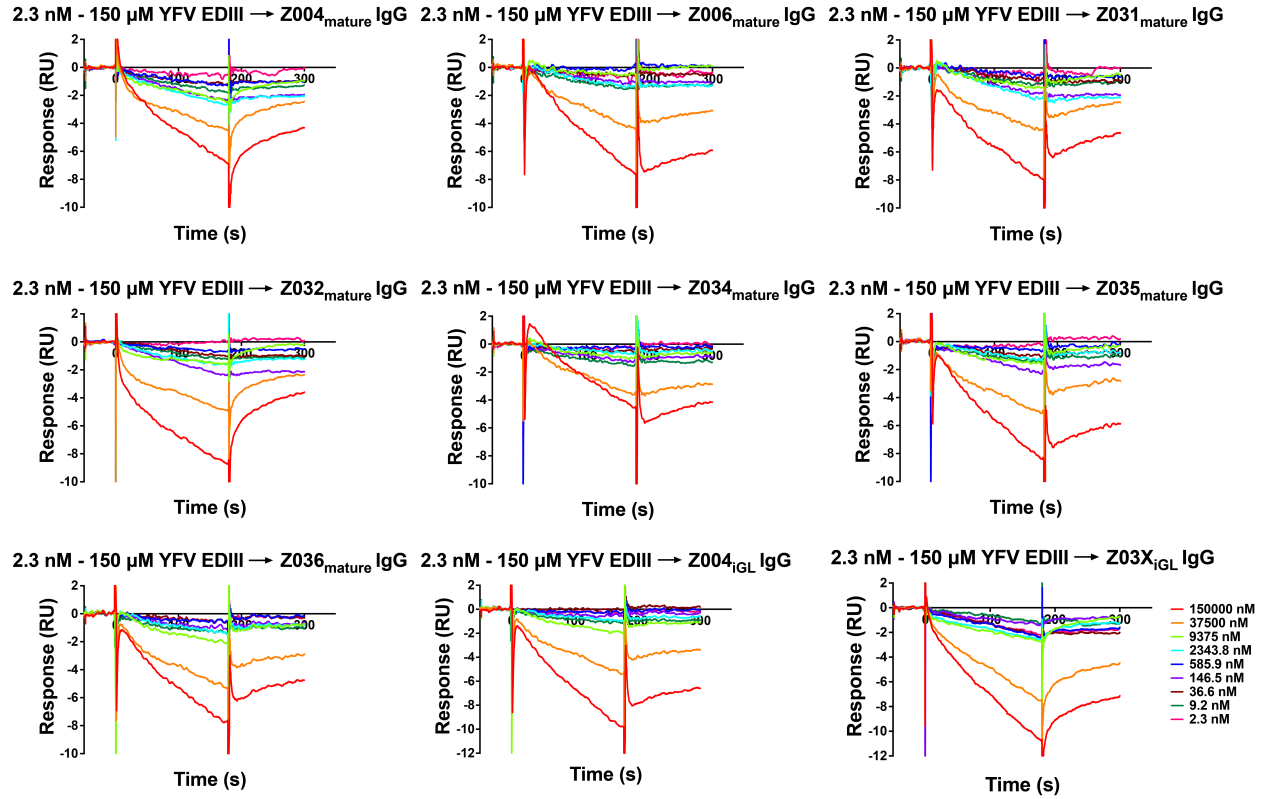


Figure S12. SPR binding assays for mature and iGL IgG (ligands) interacting with YFV EDIII (analyte). IgGs were captured on a protein A biosensor chip, and the indicated concentrations of YFV EDIII were injected. Sensorgrams are indicated in colors representing different injected concentrations. No detectable binding was found for any IgGs at YFV EDIII concentrations ≤ 150 μ M. Note that negative values indicate more YFV EDIII binds to the reference flow cell, HIV-1 IgG N6, than to the mature and iGL anti-ZIKV IgGs. Y-axes show response units (RU). Nine independent injections were performed.

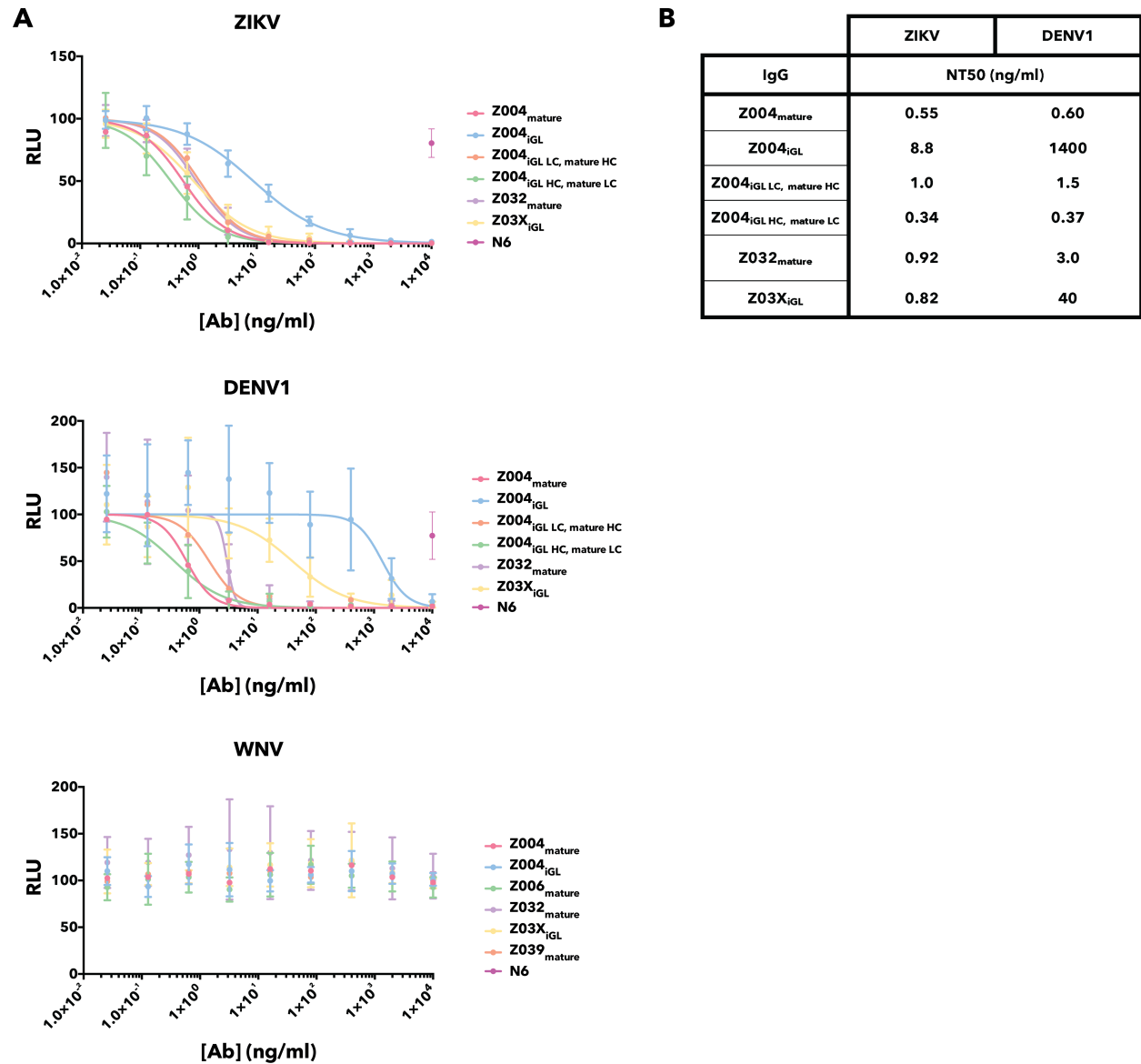


Figure S13. RVP-based neutralization assays. **A.** Neutralization curves for Abs against ZIKV, DENV1 and WNV RVPs. Two or three independent experiments were performed and are plotted on the same graph. Y-axes show luciferase activity (relative light units, RLUs) normalized to RVP luciferase activity without Ab present. The HIV-1 Ab N6 (negative control) was assessed at the highest concentration (10 μ g/ml). **B.** NT50s for Abs against ZIKV and DENV1 RVPs.

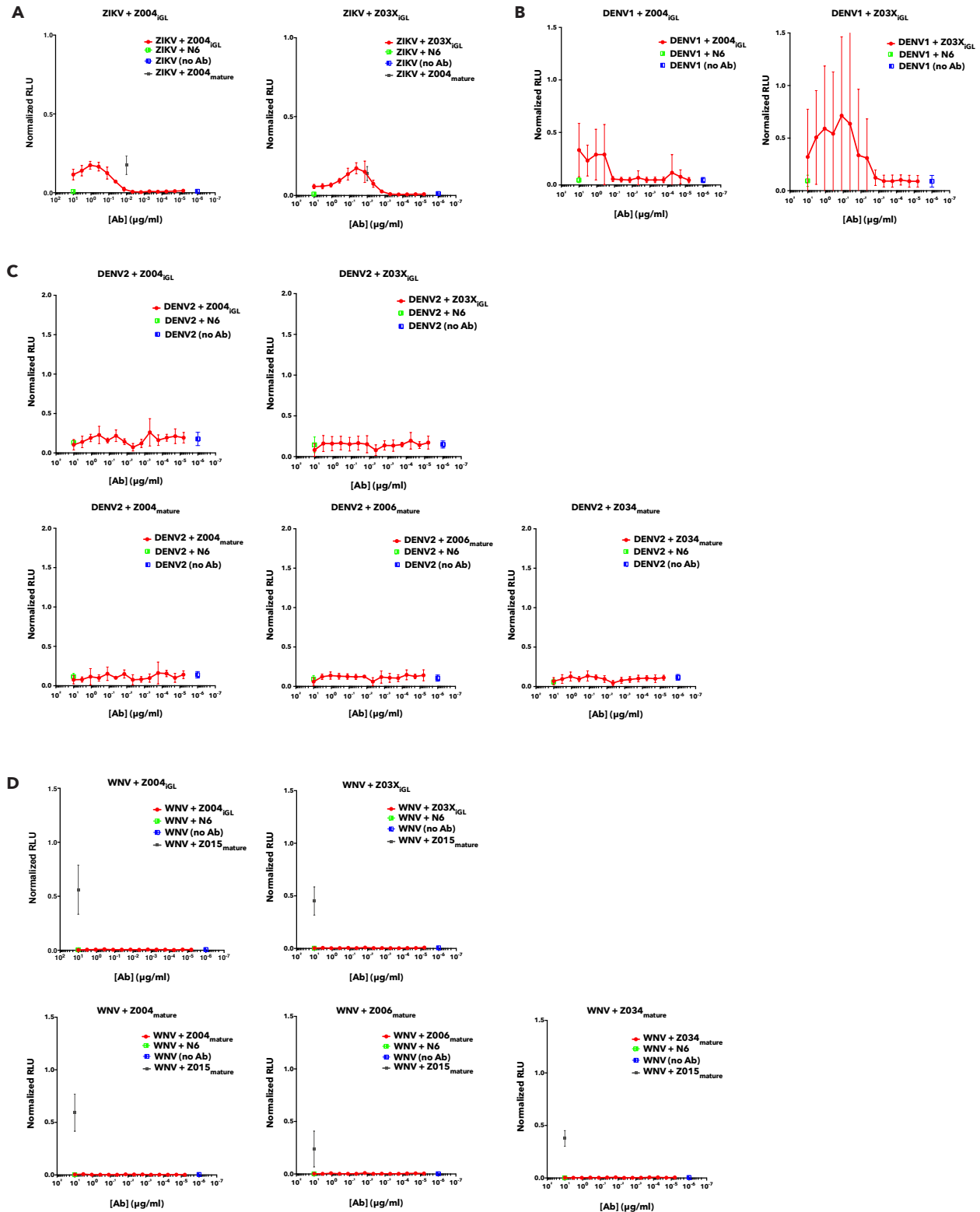


Figure S14. RVP-based ADE assays for Abs against **A.** ZIKV, **B.** DENV1, **C.** DENV2, and **D.** WNV RVPs. Two independent experiments were performed and the normalized data were combined. Data plotted is the luciferase activity (relative light units, RLUs) normalized to respective RVP luciferase activity determined on fully permissive Huh-7.5 cells (positive

control). The HIV-1 Ab N6 (negative control) and cross-reactive Ab Z015_{mature} (WNV positive control) were assessed at the highest concentration (10 µg/ml). Z004_{mature} Ab (ZIKV positive control) was assessed at 0.01 µg/ml, a concentration known to show ADE for ZIKV.

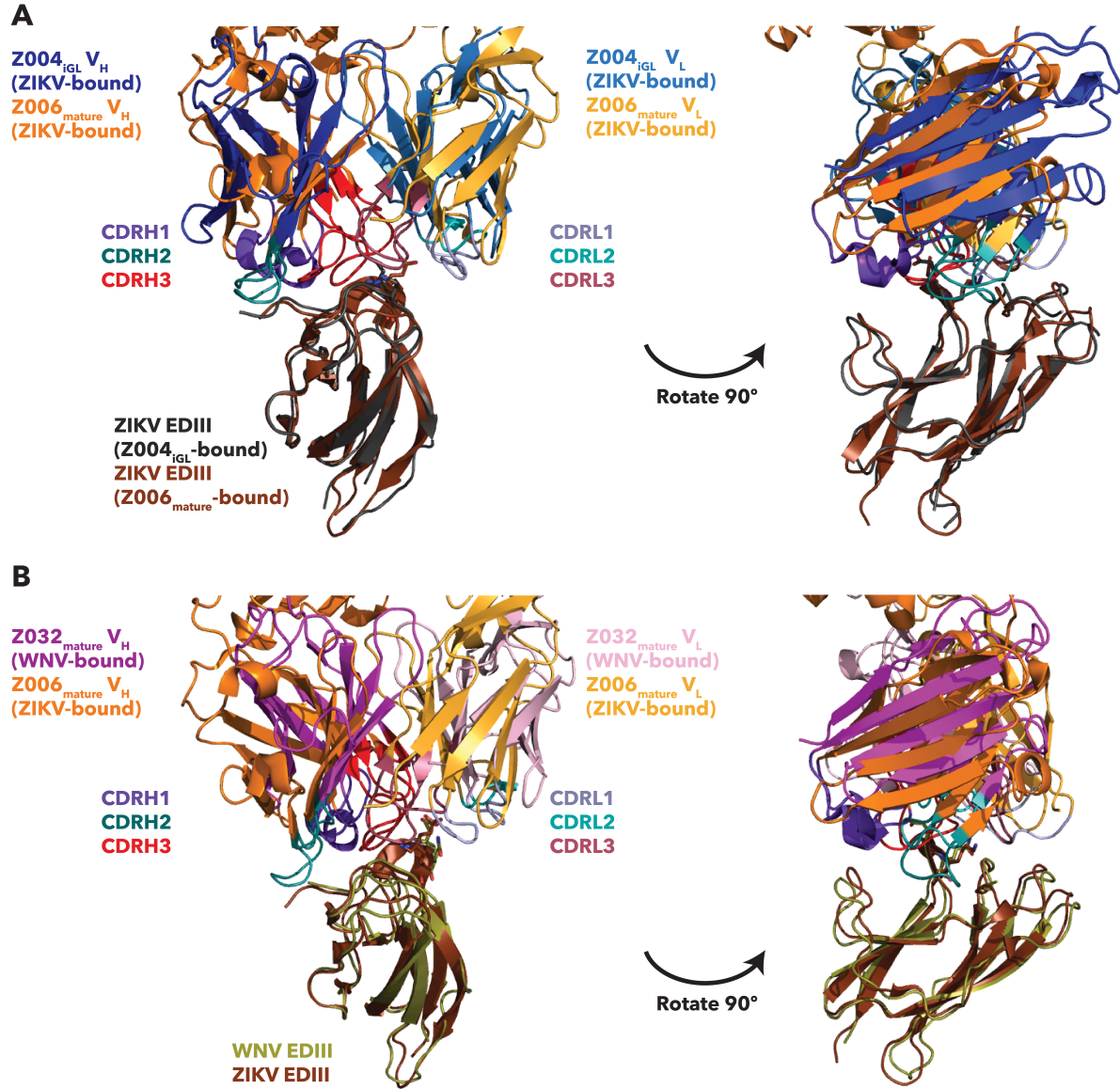


Figure S15. Recognition of EDIIIs by mature and iGL Fabs. Fab V_H-V_L-EDIII structures are shown as cartoon representations. The Fab C_H-C_L domains were truncated in the figure in order to focus on the V_H-V_L interaction with EDIII. Structures were superimposed on the EDIII. The ZIKV EK and WNV EQ motifs are shown as sticks. **A.** Superimposition of Z004_{iGL} V_HV_L-ZIKV EDIII (Figure 4A) and Z006_{mature}-ZIKV EDIII structures. **B.** Superimposition of Z006_{mature}-ZIKV EDIII and Z032_{mature}-WNV EDIII structures.

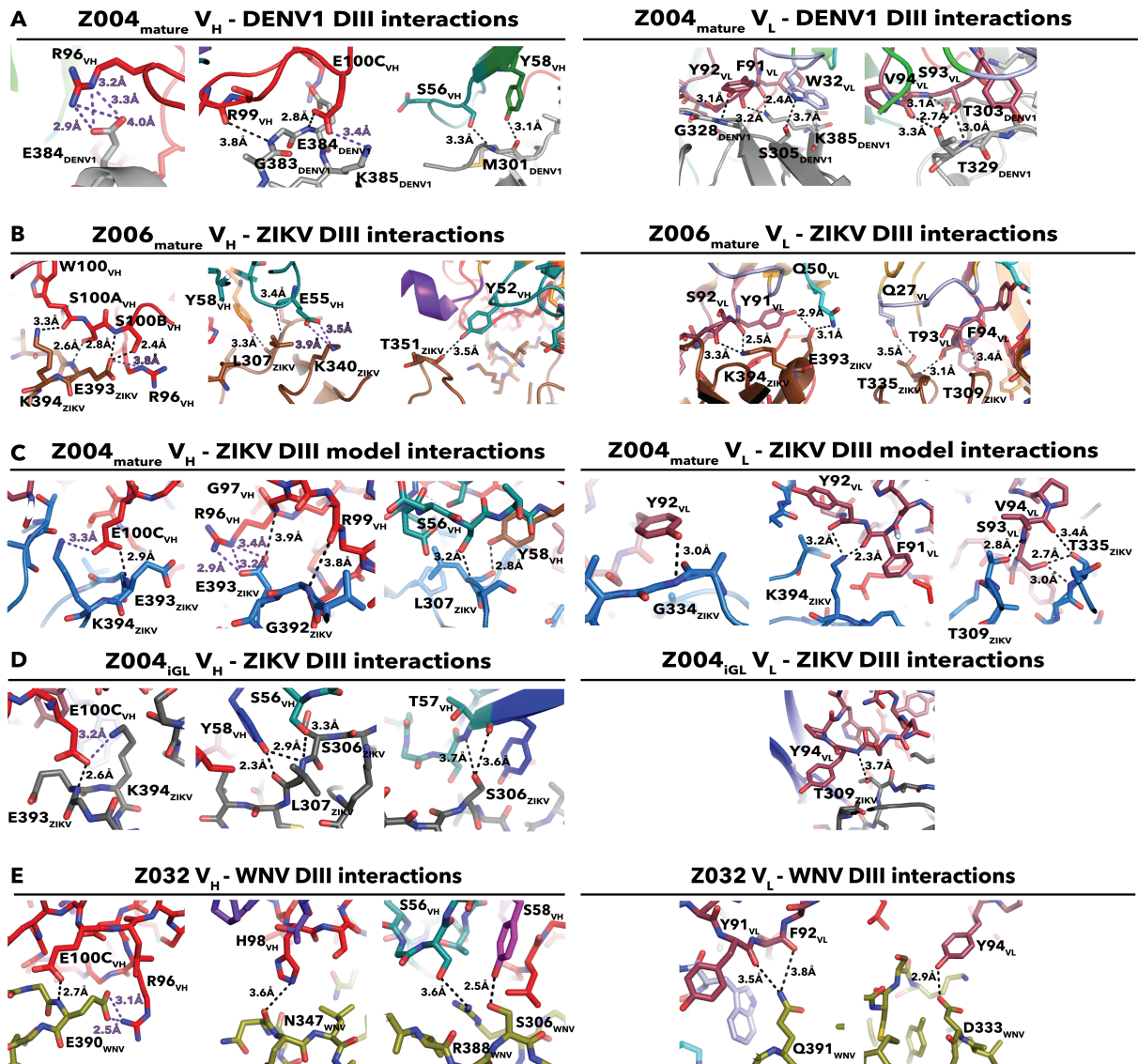


Figure S16. Close-up view of Fab–EDIII interactions. Interactions with V_H are shown on the left and interactions with V_L are shown on the right. **A.** Z004_{mature}–DENV1 EDIII crystal structure (PDB 5VIC). **B.** Z006_{mature}–ZIKV EDIII crystal structure (PDB 5VIG). **C.** Z004_{mature}–ZIKV EDIII homology model. **D.** Z004_{iGL}–ZIKV EDIII crystal structure (PDB 6UTA). **E.** Z032_{mature}–WNV EDIII crystal structure (PDB 6UTE).

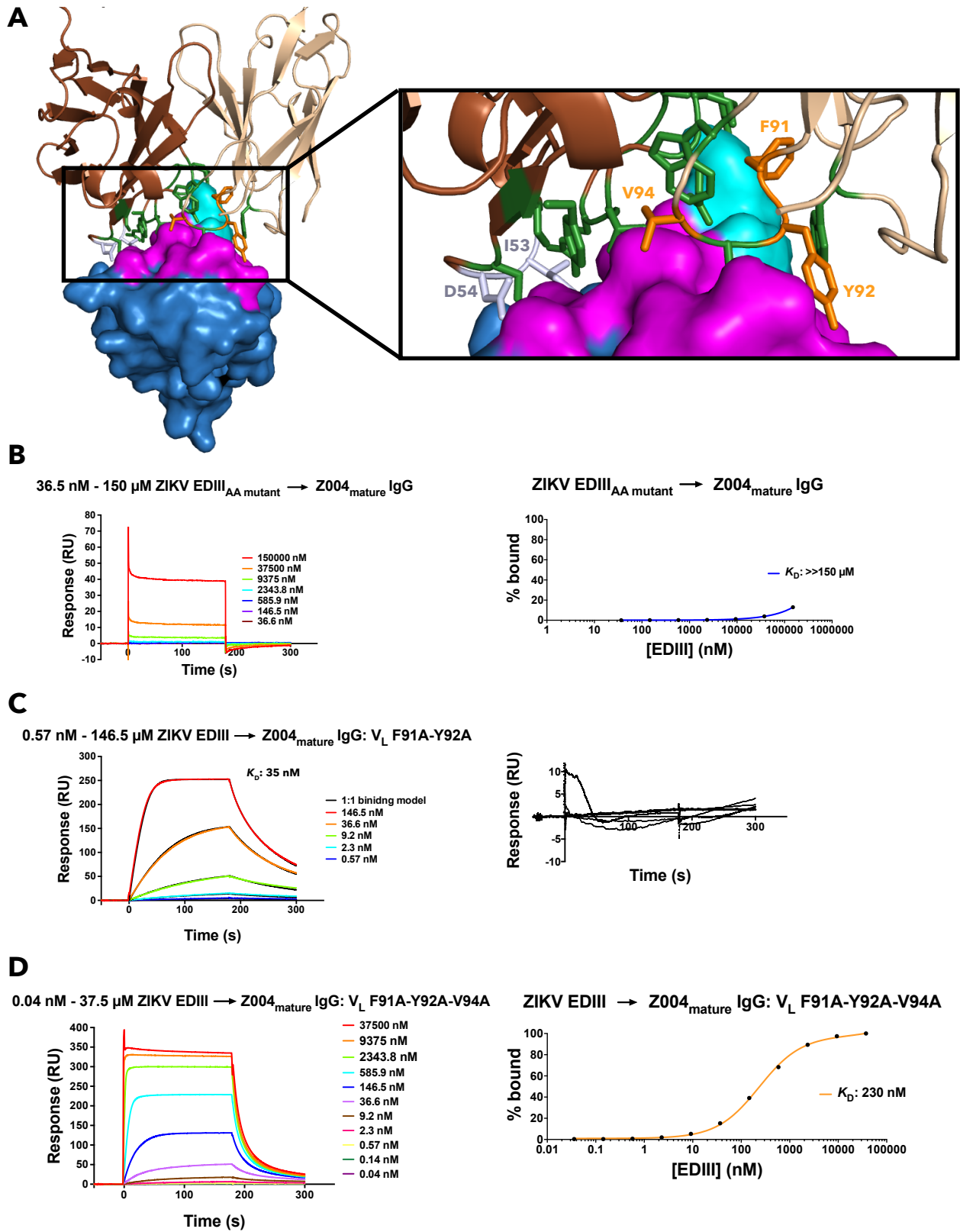
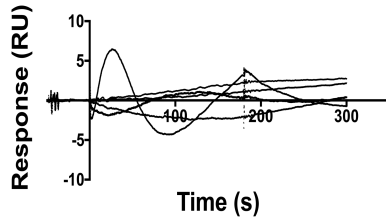
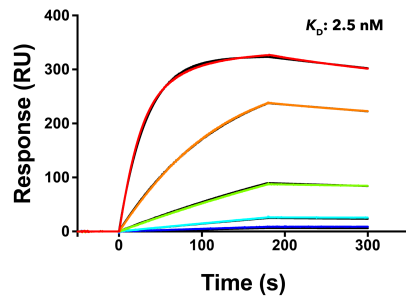


Figure S17. Comparison of ZIKV EDIII binding to Z004_{mature} IgGs with site-directed mutations in the V_L. **A.** Z004_{mature} V_HV_L-ZIKV EDIII homology model showing residues that differ between iGL

and mature Z004 Fab at the EDIII-binding interface. Z004_{mature} V_HV_L (dark brown, V_H; tan, V_L) is shown as a cartoon representation and ZIKV EDIII (dark blue) is shown as a surface representation. EDIII residues within 4 Å of the V_HV_L are shown in magenta with the EK motif in teal. V_HV_L residues within 4 Å of the EDIII are shown in dark green with the V_H residues that differ between Z004_{iGL} and Z004_{mature} (but do not interact with EDIII), I53 and D54, shown in light purple and the V_L residues that differ between Z004_{iGL} and Z004_{mature} (and interact with EDIII), F91, Y92, and V94, shown in orange. **B-D.** SPR binding assays. IgGs were captured on a protein A biosensor chip, and the indicated concentrations of EDIII were injected. Sensorgrams are indicated in colors representing different injected concentrations. Y-axes show response units (RU). Five to 11 independent injections were performed. **B.** ZIKV EDIII_{AA mutant} (E393A-K394A) binding to Z004_{mature} IgG. **C-D.** ZIKV EDIII binding to Z004_{mature} IgGs with alanine mutations in the V_L: F91A-Y92A (C) and F91A-Y92A-V94A (D). **C.** Fits to a 1:1 binding model are in black; since the model fits very closely to the data, the models are only slightly visible. The corresponding residual plot is also shown. **B,D.** Normalized equilibrium binding response (R_{eq}) from the sensorgram is plotted versus the log of the concentration of the indicated injected proteins with the best fit binding curve to the experimental data points shown as a continuous line. The standard error of the fit for the 230 nM K_D was 12 nM, with a 95% confidence of 200-260 nM for ZIKV EDIII → Z004_{mature} IgG: V_L F91A-Y92A-V94A. Since the ZIKV EDIII → Z004_{mature} IgG binding reaction did not reach equilibrium, the K_D is approximated as greater than the highest concentration of analyte injected.

0.57 nM - 146.5 nM ZIKV EDIII → Z004_{iGL HC, mature LC} IgG



0.57 nM - 146.5 nM ZIKV EDIII → Z004_{iGL LC, mature HC} IgG

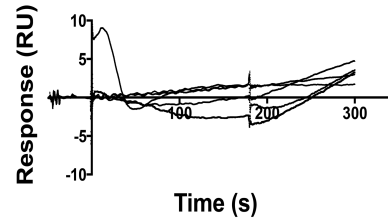
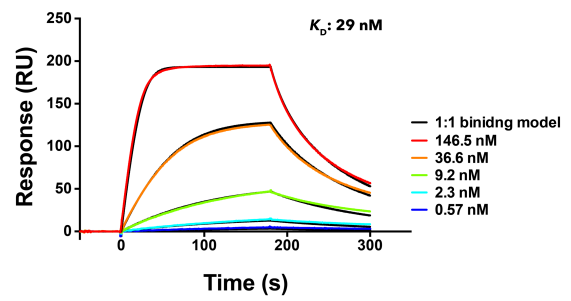


Figure S18. SPR binding sensorgrams (top; colored traces) and residual plots (bottom) for binding model fittings (top, black lines) of Z004 mature-iGL IgG chimeras (ligand) interacting with ZIKV EDIII (analyte). IgGs were captured on a protein A biosensor chip, and the indicated concentrations of ZIKV EDIII were injected. Sensorgrams are shown in colors representing different injected concentrations. Y-axes show response units (RU). Two independent experiments were performed.

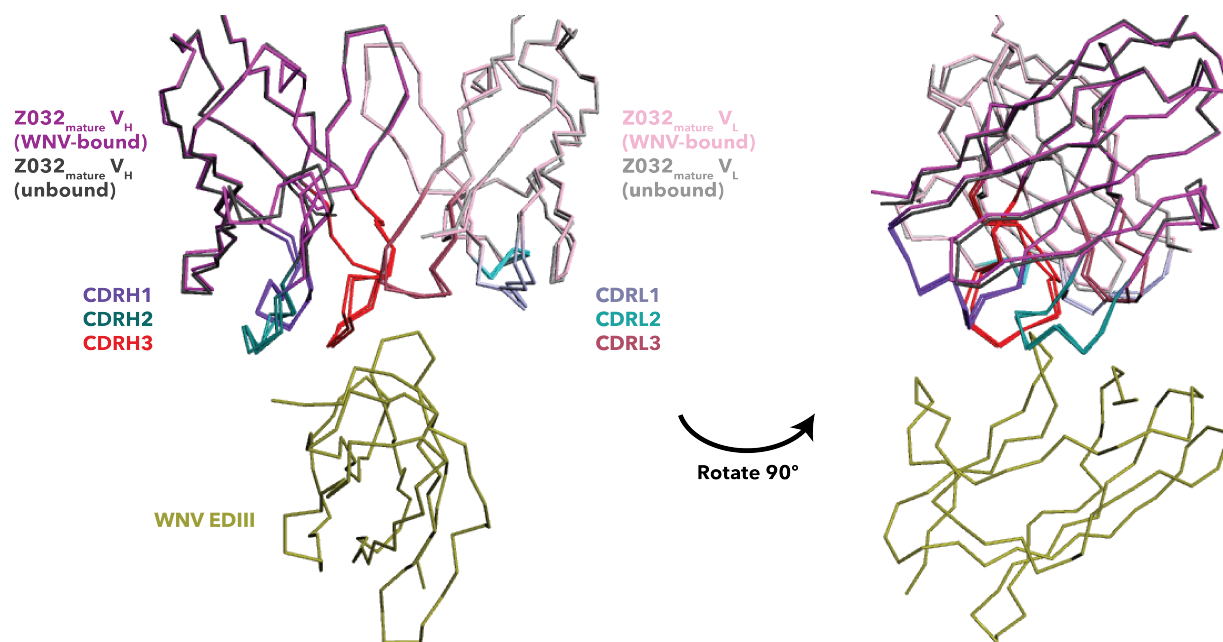


Figure S19. Comparison of bound and unbound structures of Z032_{mature}. **A.** Superimposition of V_H-V_L domains from the structure determination of a Z032_{mature} Fab–WNV EDIII complex, in which the crystallographic asymmetric unit contained both bound (chains C and D) and unbound (chains E and F shown here) Fabs.

Table S1. X-ray diffraction data and refinement statistics for Fab-EDIII crystal structures.		
	Z004_{IGL} Fab–ZIKV EDIII PDB 6UTA	Z032_{mature} Fab–WNV EDIII PDB 6UTE
Data collection		
Space group	P4 ₃ 2 ₁ 2	P2 ₁
Cell dimensions		
<i>a</i> , <i>b</i> , <i>c</i> (Å)	85.91, 85.91, 327.46	96.23, 114.02, 127.26
α , β , γ (°)	90, 90, 90	90, 109.5, 90
Resolution (Å)	38.4-3.1	40.0-2.9
<i>R</i> _{pim} (%)	8.8 (49.1) *	9.5 (50.4)
<i>I</i> / σ (<i>I</i>)	9.1 (1.7)	5.6 (1.5)
Completeness (%)	93.6 (71.4)	99.7 (99.5)
Redundancy	12.4 (7.5)	3.1 (3.1)
CC(1/2)	0.99 (0.76)	0.98 (0.64)
Refinement		
Resolution (Å)	38.4-3.1	39.9-2.9
No. reflections	21817 (1618)	57390 (5694)
<i>R</i> _{work} / <i>R</i> _{free}	27.4/29.3	22.3/26.5
No. atoms		
Peptide	8060	17255
Ligand	0	30
Water	0	0
B-factors		
Peptide	62.8	58.4
Ligand	0	80.1
Water	0	0
R.M.S. deviations		
Bond lengths (Å)	0.013	0.002
Bond angles (°)	1.56	0.55
Ramachandran statistics		
Ramachandran favored (%)	90.4	95.9
Ramachandran allowed (%)	7.54	3.97
Ramachandran outliers (%)	2.10	0.18
Number of TLS Groups	6	11

Each structure was derived from a single crystal.

*Highest resolution shell statistics shown in parentheses.

Table S2. Interacting residues in mature and iGL Fab–EDIII complexes*.

	V_H residues that interact with EDIII	V_L residues that interact with EDIII	EDIII residues that interact with Fab
Z004_{iGL} Fab – ZIKV EDIII	S56 _{VH} (CDRH2) T57 _{VH} (CDRH2) Y58 _{VH} (FWRH3) E100C _{VH} (CDRH3)	Y94 _{VL} (CDRL3)	S306 _{ZIKV} L307 _{ZIKV} T309 _{ZIKV} E393 _{ZIKV} K394 _{ZIKV}
Z004_{mature} Fab – ZIKV EDIII (homology model)	S56 _{VH} (CDRH2) Y58 _{VH} (FWRH3) R96 _{VH} (CDRH3) G97 _{VH} (CDRH3) R99 _{VH} (CDRH3) E100C _{VH} (CDRH3)	F91 _{VL} (CDRL3) Y92 _{VL} (CDRL3) S93 _{VL} (CDRL3) V94 _{VL} (CDRL3)	L307 _{ZIKV} T309 _{ZIKV} G334 _{ZIKV} T335 _{ZIKV} G392 _{ZIKV} E393 _{ZIKV} K394 _{ZIKV}
Z004_{mature} Fab – DENV1 EDIII	S56 _{VH} (CDRH2) Y58 _{VH} (FWRH3) R96 _{VH} (CDRH3) R99 _{VH} (CDRH3) E100C _{VH} (CDRH3)	W32 _{VL} (CDRL1) F91 _{VL} (CDRL3) Y92 _{VL} (CDRL3) S93 _{VL} (CDRL3) V94 _{VL} (CDRL3)	M301 _{DENV1} T303 _{DENV1} S305 _{DENV1} G328 _{DENV1} T329 _{DENV1} G383 _{DENV1} E384 _{DENV1} K385 _{DENV1}
Z006_{mature} Fab – ZIKV EDIII	Y52 _{VH} (CDRH2) E55 _{VH} (CDRH2) Y58 _{VH} (FWRH3) R96 _{VH} (CDRH3) W100 _{VH} (CDRH3) S100A _{VH} (CDRH3) S100B _{VH} (CDRH3)	Q27 _{VL} (CDRL1) Q50 _{VL} (CDRL2) Y91 _{VL} (CDRL3) S92 _{VL} (CDRL3) T93 _{VL} (CDRL3) F94 _{VL} (CDRL3)	L307 _{ZIKV} T309 _{ZIKV} T335 _{ZIKV} K340 _{ZIKV} T351 _{ZIKV} E393 _{ZIKV} K394 _{ZIKV}
Z032_{mature} Fab – WNV EDIII	S56 _{VH} (CDRH2) Y58 _{VH} (FWR3) R96 _{VH} (CDRH3) H98 _{VH} (CDRH3) E100C _{VH} (CDRH3)	Y91 _{VL} (CDRL3) F92 _{VL} (CDRL3) Y94 _{VL} (CDRL3)	S306 _{WNV} D333 _{WNV} N347 _{WNV} R388 _{WNV} E390 _{WNV} Q391 _{WNV}

*The Kabat numbering scheme was used.

Table S3. K_{DS} (nM) of Z004 Ab (mature, iGL, chimeras, and mutants) binding to ZIKV EDIII determined by SPR.

IgG	EDIII	K_D (nM)
Z004_{mature}	ZIKV	0.28
Z004_{iGL}	ZIKV	1200
Z004_{iGL LC, mature HC}	ZIKV	29
Z004_{iGL HC, mature LC}	ZIKV	2.5
Z004_{mature}	ZIKV _{AA mutant}	>>150 μ M
Z004_{mature}: V_L F91A-Y92A	ZIKV	35
Z004_{mature}: V_L F91A-Y92A-V94A	ZIKV	230

Table S4. Pairwise superimpositions and rmsd calculations of bound and unbound V_HV_L chains in the Z032_{mature}–WNV EDIII crystal structure.

Z032_{mature} V_HV_L chains: Set 1	Z032_{mature} V_HV_L chains: Set 2	Cα atom count	rmsd (Å)
C and D (bound)	A and B (unbound)	213	0.42
C and D (bound)	E and F (unbound)	210	0.45
C and D (bound)	G and H (unbound)	217	0.35
C and D (bound)	I and J (unbound)	211	0.42
A and B (unbound)	E and F (unbound)	203	0.22
A and B (unbound)	G and H (unbound)	204	0.32
A and B (unbound)	I and J (unbound)	206	0.39
E and F (unbound)	G and H (unbound)	210	0.37
E and F (unbound)	I and J (unbound)	212	0.47
G and H (unbound)	I and J (unbound)	214	0.41

References

1. Robbiani, D. F. *et al.* Recurrent Potent Human Neutralizing Antibodies to Zika Virus in Brazil and Mexico. *Cell* **169**, 597-609.e11 (2017).